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BACTERIAL INFECTION

WITH SPECIAL REFERENCE TO DENTAL PRACTICE

BY

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TO
MY FATHER

63341

PREFACE.

THIS book is devised for readers desiring one or both of two things: (1) An adequate conception of infection and (2) a comprehensive understanding of the bearing of infection on the problems of dental practice. It would be absurd to imagine that these desiderata can be realized in full merely by the study of books. Clinical and laboratory experience is necessary. But the existence of such a book as this may be justified if it facilitates the full realization and if by its very shortcomings it emphasizes the need for further investigation.

Anyone who is professionally concerned with human disease needs an adequate and balanced picture of infection. Although the development of such a picture should be the central purpose of any course in medical bacteriology, the writer is unaware of any book, suitable for the beginner, which attempts to cover this field as an entity. The subject matter is considered, scattered in the classical works on bacteriology, on hygiene, on clinical pathology, on general pathology, on diagnosis and on clinical medicine. The bringing together of the facts and ideas, which have been hitherto treated in different and separate divisions of medical science, into the natural and logical relations which they possess when regarded as parts of the general subject of infection—has been the chief aim of this book. Because of the great relative importance of bacteria in human disease, attention has been in the following pages almost exclusively given to *bacterial* infection. But occasional reference has been made to other forms of microorganisms where they seemed to suit my purpose better. Throughout this book the thought has been constantly in mind that what the prospective practitioner of medicine or dentistry needs from a course in bacteriology is information as to how bacteria produce disease and how the host is affected by, and responds to, the presence of bacteria and their products.

The dental phases of these pages, particularly in Part III, it is hoped, will not only interest the student and practitioner of dentistry

but also will have an appeal to the general practitioner and internist who is willing to admit that infection in one part of the body may influence the general condition of the patient or the health of other, more or less remote, parts. Fortunately today it is unnecessary to convince the dental profession that a knowledge, equivalent to that possessed by the physician, of what infection means is essential for intelligent and honest practice. This standpoint is accepted as axiomatic. An introductory sentence to an interesting, early paper on oral bacteriology by Roughton (*Trans. Odont. Soc. Great Britain*, 1893 *n.s.* 25, *p.* 71) seems at least by its implications to tell almost the whole story. "If there were no microorganisms in the mouth there would be no decay of teeth, and if there were no decay of teeth there would be very few dentists"

Following the Caesarean tradition, I have divided the subject matter considered into three parts. Part I is intended to acquaint the reader with a certain minimum of information upon the morphology, physiology and ecology of the bacteria. Sterilization by chemicals and heat is treated here. In Part II the attempt has been made to treat the subject of infection as an entity. In Part III every effort was made to present a full summary and a critical analysis of what is known about the common infections peculiar to, or peculiarly associated with, the oral cavity. I wish to take this opportunity to thank Dr. Hermann Prinz in whose library I have found many of the German and Austrian contributions to oral bacteriology, which otherwise I could not have consulted in the original.

Throughout the work the general plan has been to present in logical sequence what seemed to be salient and significant data and ideas, and then to present some of the evidence therefor, to illustrate them and to point out certain qualifying circumstances. Except in Part III I have not attempted consistently to trace the historical development of the dominant hypotheses and concepts of infection. A careful effort has been made to cite accurately the original references. This is done because there are few things which exasperate me more than to see a statement made without at the same time being enabled to refer to the original and full report of the evidence; and because it is desirable to encourage and facilitate the consultation of the literature by the student and practitioner of dentistry.

J. L. T. A., JR.

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BACTERIAL INFECTION.

PART I.

BACTERIOLOGY.

CHAPTER I.

BACTERIA.

BACTERIA are minute, unicellular, vegetable, organisms, devoid of chlorophyl or other photo-synthetic pigment, reproducing by a process of binary fission, and in shape, spherical or spheroidal, straight or curved rods, spirally wound filaments, and long, branched and unbranched filaments. Like all "definitions," at least in biological fields, the various characteristics require qualification, exposition or amplification if misconceptions are to be avoided.

The recognition that bacteria are living beings is so general that it is usually taken for granted. Some of the evidence supporting this view may be summarized as follows: (1) Bacteria are self-perpetuating, that is they can transform inert material of their environment into their own protoplasm and they reproduce after their kind; (2) they consume oxygen and liberate carbon dioxid in definite proportions; (3) they liberatè heat; (4) they produce that type of catalysts known as enzymes, which are analytic and synthetic in function. ✓

MORPHOLOGY OF BACTERIA.

The unit of microscopical measurement, applicable to bacteria, is the micron (μ) which is 0.001 mm. and approximately equivalent to $\frac{1}{25000}$ of an inch. The majority of pathogenic spherical bacteria exhibit a diameter not varying far from 1 μ . Among the rod-

shaped forms, for example, the dimensions of the influenza bacillus are about 0.5 to $2\ \mu$ by 0.2 to $0.3\ \mu$, of the typhoid bacillus 1 to $1.5\ \mu$ by 0.6 to $0.8\ \mu$, of the anthrax bacillus 5 to $10\ \mu$ by 1 to $1.25\ \mu$, and of the vibrio of Asiatic cholera 2 by $0.4\ \mu$. The spiral of syphilis is 6 to $14\ \mu$ by 0.25 to $0.3\ \mu$. The branched and unbranched filamentous forms are of rather indeterminate length, often exceeding the diameter of a microscopical field under oil immersion.

The minute size makes difficult the recognition of cellular structure. The bacterial cell differs in many respects from the classical cell of botany and zoölogy. No morphological nucleus is demonstrable although the nucleoprotein content is high. It is likely that the chromatin, the distinctly nuclear material, is diffusely scattered through the cytoplasm. This substance is usually homogeneous, although some species constantly show metachromatic granulations or barrings, and under certain conditions it may be vacuolated.

The wall of the bacterial cell is possessed of a high degree of rigidity. This is evident from the resistance offered to change of form by variation in osmotic or atmospheric pressures.



FIG. 1.—*Bacillus chauvei*, spore formation. $\times 1000$. (Kendall.)

Endospores.—In some species under certain conditions there is formed within the cytoplasm, a spherical or ovoid, glistening, refractile body, staining with difficulty. This is the endospore. Its chief or only function is to act as a resting stage during which the bacterium is more resistant to unfavorable environmental conditions than in the ordinary vegetative stage. The endospore is liberated by the breaking down of the cell wall. Upon the recurrence of favorable conditions the endospore germinates and a new cell body is assumed. Usually but a single endospore is formed

within each bacterial cell but sometimes appearances very suggestive of two endospores within a single cell are encountered. If this be true and if both are capable of germination, the endospore is a potentially reproductive body. This mode of reproduction, if it actually exist, is however of relatively little importance among the bacteria. The endosporogenous, pathogenic forms include *B. anthracis*, *B. tetani*, *B. welchii* and the other organisms of gas gangrene.

Motility.—Some bacterial species exhibit a true motility. This is accomplished, with the exception noted below, by means of long, delicate, whip-like, vibratile, protoplasmic processes (flagella) extending out from the cell periphery. If the spirochetes be regarded as bacteria (and there is some doubt as to the correctness

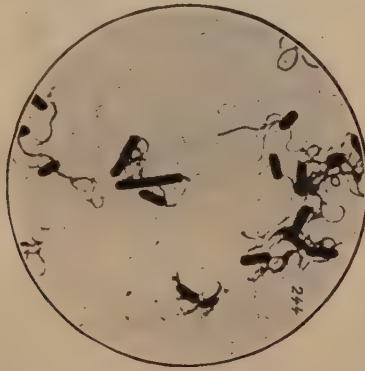


FIG. 2.—*Bacillus coli* flagella. $\times 1500$. (Kolle and Hetsch.)

of this viewpoint), they form an exception because an undulating membrane has been described for some members of this group and others are motile with no demonstrable flagella.

Capsules.—The bacterial cell of some species is surrounded by a mucoid capsule. Toennissen¹ reports that the capsule of Friedländer's bacillus is galactan, a polysaccharid hydrolizing to galactose. Among the pathogenic forms this structure is to be seen solely or usually when the organism is living in the infected animal, in pathological discharges and exudates, or in artificial media containing native (unchanged by heat or chemicals) proteins. The presence of a capsule often seems to be correlated with virulency: *e. g.*, virulent pneumococci are encapsulated, in avirulent strains the capsule is absent, and in what is apparently the most virulent strain,

¹ Centralbl. f. Bakteriöl., 1920, 85, 225.

Type III, the capsule formation is most marked. Encapsulated bacteria make poor antigens. It has been suggested that the capsule represents a defensive reaction on the part of the bacterial cell, protecting it from antagonistic factors present or developed in the host. Encapsulated forms are commonly exhibited by the pneumococci, *Micrococcus tetragenus*, the anthrax bacillus, Friedländer's pneumobacillus and its closer relatives.



FIG. 3.—Pneumococcus showing capsules. (Kendall.)

Taxonomy.—The vegetable nature of the bacteria has been and still is frequently and seriously questioned. A final answer is not possible at the present day, nor does it appear to be of much import to the student of disease. It is quite possible that the bacteria may be included in Haeckel's kingdom of the Protista, that is a group neither animal nor plant but the most primitive group of living forms from which animals and plants were evolved and diverged, but within which such separation had not occurred. The author is inclined to regard the affinities of the bacteria to the plants as the closer; while the affinities of the spirochetes to the protozoa appear the closer. It is difficult to believe that the branched filamentous forms are not closely related to indubitable fungi on the one hand and on the other all grades of transition are found between the branched filamentous forms and all other types of bacteria. Of the spirochetes it may be said that the possession of an undulating membrane and of non-flagellar motility and the occurrence of reproduction by longitudinal, instead of transverse, splitting are characteristics which would be unique among the bacteria but which are common among the protozoa.

Reproduction.—Reproduction among the bacteria is always asexual. It is typically accomplished by a single cell dividing into two equal daughter cells. The plane of division in the spherical forms is the equatorial plane. In the other forms, if we except the spirochetes which sometimes show longitudinal splitting, the plane passes at right angles to the longer axis (binary fission). Among the branched filaments, coccoid forms are produced by the repeated passage of planes of division at right angles to the long axis. These coccoid forms are reproductive bodies, each one capable of developing into a new filament. The possible role of the endospore as a reproductive form has been mentioned above.

The arrangement of the bacterial cells into groups is largely determined by direction of the planes of division and is at times very characteristic. This is particularly so among the spheroidal forms, where a grouping in pairs is designated by the term *diplococcus*; in irregular plates, *staphylococcus*; like a string of beads or in chains, *streptococcus*; and in fours as at each corner of a square, *tetracoccus*.

PHYSIOLOGY OF BACTERIA.

Bacteria, like all other forms of life, are sensitive to certain environmental factors, a few of which will be considered below. They respond to sudden changes in the osmotic pressure of the medium in which they are suspended by changes in size and shape, appearing as "involution forms."

Hydrogen-ion Concentration.—The hydrogen-ion concentration (pH) of the medium is of great significance for bacterial growth. The following table furnishes a summary of most of the available data on this phase of bacterial physiology.

In respect to the influence of light, the shorter the wave-length the greater is the damage done. The violet and ultra-violet part of the spectrum is the more destructive while the red and infrared, with temperature constant, are almost or quite innocuous. Direct sunlight rapidly kills vegetative bacteria, while diffuse daylight is far less energetic. Bacteria thrive best in the dark.

Roentgen-rays and radium exert little damage to the bacterial cell.

Moisture is indispensable for bacterial growth. At least a film of water around the cell is necessary for the performance of its ordinary activities. Different bacteria show quite a wide range of susceptibility to drying. The endospores resist desiccation for years. The average duration of viability in a species is an important factor in determining the dissemination of some infectious diseases.

REACTION OF CULTURE MEDIUMS FOR VARIOUS ORGANISMS IN TERMS OF pH.

Medium and organism.	Acid limit.	Alkaline limit.	Optimum.
Russell double sugar for <i>B. typhosus</i> , <i>B. paratyphosus</i> A and B, dysenteriae and coli	7.0	7.8	7.4-7.6
Endo's medium for intestinal flora ¹	...	...	7.8-8.0
Brilliant green ¹	6.4	7.2	6.8-7.0
Simple nutrient agar for typhoid ¹	4.0	9.6+	6.2-7.2
<i>B. typhosus</i> ²	6.2	7.6	6.8-7.2
<i>B. typhosus</i> ⁴	...	...	6.8-7.0
<i>B. paratyphosus</i> A ¹	4.0	9.6+	6.2-7.2
<i>B. paratyphosus</i> A ²	4.5	7.8	6.4-7.0
<i>B. paratyphosus</i> B ¹	4.0	9.6+	6.2-7.2
<i>B. paratyphosus</i> B ²	4.3	8.0	6.4-7.2
Dysentery, Shiga ¹	4.8	9.6+	6.2-8.4
Dysentery, Flexner ¹	4.8	9.6+	6.2-8.4
Dysentery, "Y" ¹¹	4.8	9.6+	6.2-8.4
Cholera vibrio ¹	5.6	9.6+	6.2-9.0
<i>M. melitensis</i> ¹	6.3	9.6	6.6-8.2
Infusion broth for pneumococcus ¹	5.0	8.0	7.8
Pneumococcus ⁵	5.1	...	6.8-7.4
<i>Streptococcus hemolyticus</i> ¹	4.5	8.0	7.6-7.8
<i>S. viridans</i> ¹	4.5	8.0	7.6-7.8
Infusion glucose agar for meningococcus ¹	7.4	7.8	7.6
Chocolate medium for <i>B. influenzae</i> ¹	?	?	7.8
<i>B. influenzae</i> (?) ²	6.2	7.6	7.0
Vedder's starch medium for gonococcus ¹	7.0	8.0	7.4-7.6
Gonococcus ²	6.0	8.3	7.3
<i>B. diphtheriae</i> ²	6.0	8.3	7.3-7.6
<i>B. tuberculosis</i> (from horse) ²	6.0	7.6	6.8-7.2
<i>B. coli communis</i> ²	4.4	7.8	6.0-7.0
<i>B. suipestifer</i> ²	5.0	8.2	7.0-7.6
<i>Ps. pyocyanea</i> ²	5.6	8.0	6.6-7.0
<i>B. vulgaris</i> ²	4.4	8.4	6.0-7.0
<i>Microspira comma</i> ²	6.4	7.9	7.0-7.4
<i>Microspira cinnabareus</i> ²	5.0	7.8	6.0-7.0
Pneumococcus (Types I, II, III) ²	7.0	8.3	7.8
<i>Streptococcus liquefaciens</i> ²	5.5	8.0	6.2-7.0
<i>B. subtilis</i> ²	4.5	8.5	6.0-7.5
<i>B. anthracis</i> ²	6.0	8.5	7.0-7.4
<i>B. anthracoides</i> ²	6.0	7.8	6.8-7.2
<i>B. pestis</i> ²	5.6	7.5	6.5-7.1
<i>B. pestis</i> ⁶	5.0	6.2-7.0	8.2
<i>B. pestis</i> ⁶	5.4	6.0-6.6	7.6
<i>B. prodigiosus</i> ²	5.0	8.0	6.0-7.0
ANAEROBES.			
<i>B. tetani</i> ²	5.5	8.3	7.0-7.6
<i>B. sporogenes</i> ^{2,3}	5.0	8.5	6.0-7.6
<i>B. histolyticus</i> ^{2,3}			
<i>B. canadiensis</i> ^{2,3}			
<i>B. putrificiens</i> ^{2,3}			
<i>B. perfringens</i> ^{2,3}			

¹ Fennel, Eric A., and Fisher, Maud B.: Jour. Infect. Dis., 1919, 25, 451.

² Dernby, K. G.: Ann. de l'Inst. Pasteur, 1921, 35, 277.

³ It is extremely difficult to isolate these anaerobes in pure culture (vd. Hall, Ivan: Jour. Bacteriol., 1921, 6, 1). The unanimity of these pH values might be due to the mutually equalizing effect of mixed cultures.

⁴ Schoenholtz and Meyer: Jour. Infect. Dis., 1921, 28, 384.

⁵ Lord, Frederick T., and Nye, Robert N.: Jour. Exper. Med., 1922, 35, 687.

⁶ D'Aunoy, Rigney: Jour. Infect. Dis., 1923, 33, 391.

Temperature.—In respect to heat or thermal energy, the biological generalization holds good that within certain limits an increase of temperature increases the metabolic rate. It is customary to recognize four crucial points or rather ranges for each species; the minimum, optimum and maximum ranges and the thermal death-point. The minimum point is the temperature below which growth and reproduction will not occur. Bacteria are far less sensitive to exposure to low temperatures than to high. The optimum point or range is the temperature which is most favorable for growth and reproduction. The maximum point is the temperature above which growth and reproduction does not occur although the cell remains viable. Growth of endosporogenous bacteria at temperatures near the maximum may prevent the development of the endospore and successive cultures from this strain may fail to regain that function. The virulency of pathogenic forms is likewise diminished or lost by cultivation at temperatures above the optimum. On the basis of minimum, optimum and maximum temperatures bacteria are divided into three groups—psychrophilic, mesophilic and thermophilic. The following chart shows the characterization of these groups.

	Minimum.	Optimum.	Maximum.
Psychrophilic	0° to 5° C.	15° to 20° C.	30° C.
Mesophilic	10° to 15° C.	35° to 38° C.	39° to 44° C.
Thermophilic	40° to 49° C.	50° to 55° C.	60° to 70° C.

Only mesophilic bacteria are pathogenic for man. The temperature of the host must fall within the optimum temperature range of a bacterial species, if the latter is to be pathogenic for the former. This, of course, does not mean that such coincidence alone determines pathogenicity; but it does mean that psychrophilic and thermophilic bacteria are not pathogenic, in the narrow sense of this word for man. Many mesophilic bacteria, of course, are not pathogenic for man. The optimum temperature requirements of a bacterial species limits the number of possible host-species. This simple fact may explain some instances of natural immunity, *e. g.*, the resistance of birds, which have the higher temperature, to many mammalian infections and *vice versa* or the resistance of cold-blooded animals to many infections among the warm-blooded and *vice versa*.

In a now classical experiment Pasteur succeeded in infecting the domestic fowl, which normally is highly resistant, with anthrax. The hen inoculated with *B. anthracis* was kept for some time in a bath, the water covering one-third of the body, to lower the bird's

temperature. When treated in this way, the hen dies. A mere fall of temperature from 42° C. (normal for these birds) to 38° C. appeared sufficient to cause a receptive condition. A similar instance has been reported by Strouse.¹ The pigeon, with a normal temperature of 106° to 108° F., is highly resistant to pneumococcal infection. This temperature was reduced by the subcutaneous injection of pyramidon. Subsequent injection of pneumococci proved rapidly fatal. Findlay² confirmed these findings. The cloacal temperature of pigeons was reduced from 106.2° to 101.2° F. by 0.45 gm. of pyramidon. Then pneumococci were injected, with death resulting in thirty-one hours. A factor in the increased susceptibility to certain infections manifested during some of the avitaminoses, may be this same depression of the normal temperature.

Sterilization by Heat.—Thermal energy is widely used as a sterilizing agency. All things considered such methods are superior in simplicity and effectiveness. The vehicle for the thermal energy may be air, in which case “dry heat” is spoken of. It may be water, in which case “moist heat” is spoken of. The temperatures employed may be that of boiling water (100° C.), or below or above this point. Where temperatures above 100° C. are used, we are dealing with steam under pressure. Besides air and water as vehicles, non-aqueous liquids as the various oils and some hydrocarbons are sometimes used. At any given temperature, moist heat is decidedly more effective than either dry heat or where a non-aqueous liquid (assuming it to be chemically inert) is used.

The subject of the sterilization of dental instruments has been very intelligently considered by Hasseltine.³ In order of merit he places the methods depending upon heat as the active disinfecting agent as follows:

1. Boiling for at least ten minutes in 0.25 per cent sodium hydroxid.
2. Use of water-bath at 80° C. for at least ten minutes.
3. Use of moist heat in free chamber (Arnold sterilizer) for at least ten minutes after thermometer reaches 100° C.
4. Submersion in boiling water for at least ten minutes, the source of heat being removed immediately prior to submersion of the instrument.
5. Application of dry heat by passing instrument through a free flame.
6. Dry heat in closed chamber.

¹ Jour. Exper. Med., 1909, **11**, 743.

² Lancet, 1922, **202**, 714.

³ Hyg. Lab. Bull. No. 101, U. S. Treas. Dept., October, 1915.

His specific recommendations are given below almost *verbatim*.

1. That all instruments and appliances be rendered mechanically clean by washing in water with a brush or sponge.

2. That the following instruments and appliances be boiled or submitted to 80° C. in a slightly alkaline solution (0.25 per cent sodium hydroxid) for at least ten minutes.

Artificial teeth used in match-	Pluggers.
ing and measuring.	Pyorrhea instruments.
Broaches and their holders.	Polishing points and brushes (if not discarded after using once).
Burnishers.	Reamers.
Burrs.	Root elevators.
Chip blowers.	Rubber-dam clamps and forceps for same.
Chisels.	Rubber-dam weights and metal parts of holder.
Drills.	Saws.
Excavators.	Scalers.
Explorers.	Scissors.
Files.	Scratch wheel on head of engine.
Forceps, extracting.	Spatulas, metal.
Forceps, foil.	Syringes, hypodermic.
Hand pieces for engine.	Syringes, water.
Impression trays.	Tongue-holding forceps.
Knives and lancets.	Mirrors (if 80° bath be used, but not to be boiled).
Mallets, hand and automatic.	
Mixing slabs.	
Mouth gags.	
Mouth-piece of saliva ejector.	
Pliers.	

3. That instruments in the above list whose mechanical construction makes it difficult to remove the excess of water are to be placed in 95 per cent alcohol for ten minutes to remove water, then removed and allowed to dry.

4. That only instruments with metal handles be used by dentists desiring to follow this method.

5. That the following instruments be sterilized by immersion in 5 per cent solution of phenol for at least sixty minutes:

Mounted stones.

Tortoise-shell instruments.

Mirrors (when 80° bath is not used).

Other instruments not of metallic nature and which cannot be replaced by metallic instruments.

6. That instruments, after using, be placed in a fluid medium, preferably clean water, to avoid drying of infectious material and to facilitate their mechanical cleansing.

7. That no instrument or appliance, used on a patient directly or indirectly, be used on any other patient until recommendations 1 and 2, or 1 and 5, as the case may be, have been complied with.

The objections to moist heat are rusting and the dulling of cutting edges. In the above recommendations, the alkalization of the water is to avoid the rusting and the temperature below the boiling point (80°C.) is to minimize the dulling effect.

It is not by any means certain that complicated mechanisms as the dental hand-piece can without damage be sterilized by moist heat. I have shown¹ that it is practicable to immerse the hand-piece in an oil-bath maintained at a given temperature. Ordinary mineral or paraffin oil will do. If one is only wishing to destroy the asporogenic bacteria, 130°C. for five minutes will suffice. If one wishes to ensure the destruction of the sporogenic types as well, 185°C. for five minutes or 175°C. for ten minutes or 165°C. for fifteen minutes will suffice.

It is very important to remember in connection with all recommended sterilizing procedures that one must not slavishly follow a prescribed method. To be on the safe side in this matter it is necessary to determine the thermal death-point *under the conditions obtaining in the individual office*. Slight and even unrecognized departures from a described method may render such a modification useless.

Nature of Food Supply.—The source of food supply has, of course, been an important factor in the evolution and differentiation of the bacteria.

On this basis, these organisms may be classified as follows:

I. Autotrophic.

II. Heterotrophic.

A. Metatrophic or saprophytic.

1. Zymogenic.

2. Saprogenic.

B. Paratrophic or parasitic.

1. Non-pathogenic.

2. Pathogenic.

The *autotrophic* forms are not directly dependent upon other forms of life for their food supply. They can synthesize protoplasm and meet their energy requirements from simple, inorganic molecules; *e. g.*, CO_2 , H_2O , sulphates, nitrates, ammoniacal salts, phosphates, etc. The *heterotrophic* forms are directly dependent

¹ Dental Cosmos, August, 1924.

upon other forms of life. They require preformed carbohydrates, fats, and proteins. The *metatrophic* or saprophytic forms live on dead organic matter. In the case of the zymogenic, this dead organic matter is predominantly carbohydrate in nature and the result of the action of the bacterial enzymes thereon is fermentation, *i. e.*, the breaking down of carbohydrates with the liberation of alcohols, simple organic acids, CO_2 , methane, hydrogen, water, etc. In the case of the *saprogenic*, the dead organic material is predominantly protein in nature and the result of the action of the bacterial enzymes thereon is putrefaction, *i. e.*, the breaking down of the protein molecule with the liberation of such products as indol, skatol, H_2S , NH_3 , etc. The *paratrophic* or *parasitic* forms live on other living animals or plants.

The metatrophic and paratrophic mode of nutrition may or may not be obligatory for a given bacterial species. We can speak of an obligate or of a facultative saprophyte or parasite. As a matter of fact it may prove possible to cultivate all parasites on non-living organic substances. When we speak of an obligate parasite, we may mean nothing more than that the nutritional requirements of the bacterium are so delicate and exacting that hitherto it has been impossible to duplicate them artificially. Little or nothing is known regarding the phylogeny of the bacteria. The view which possesses considerable *a priori* consistency with our present knowledge, is that the obligate parasites were derived from facultative forms which in turn arose from the strict saprophytes.

Many substances by virtue of their chemical properties profoundly influence bacterial activities. We shall limit our discussion of these substances to oxygen and that group exerting in common a deleterious influence, known variously as disinfectants, antiseptics and germicides.

CHAPTER II.

RELATION OF BACTERIAL GROWTH TO OXYGEN SUPPLY. ANAËROBIOSIS.

OXYGEN was discovered by Priestley in 1774. A correct appreciation of its physiological significance we owe largely to Lavoisier who was guillotined during the French Terror. Pleas for clemency were officially set aside with the statement, "La Republique n'a pas besoin de savants, . . .". Oxygen, it became recognized, was indispensable for vital processes. For more than sixty years after Lavoisier's death, it was taken for granted that the oxygen necessary for such purposes must exist in molecular form, that is, as now expressed by the symbol O_2 . This molecular oxygen might exist as a gas, as in the atmosphere, or in aqueous solution where it would be available for such forms as many aquatic and marine invertebrates and fishes.

The view that only free molecular oxygen was utilizable by living forms was invalidated by Pasteur in 1861. He observed in studies on butyric fermentations that the microorganisms concerned "live and multiply indefinitely, without requiring the least quantity of air. And not only do they live without air but air actually kills them."¹ To describe organisms exhibiting this characteristic the term "anaërobes" was invented; while the forms requiring free molecular oxygen were known as aërobes. It has been found that anaërobes need oxygen for their vital processes as much as the aërobes; the essential difference being that this element is secured by the former group from reducible, oxygen-containing compounds, such as the carbohydrates. Consequently dextrose is often added to media used for anaërobic cultivation.

It is customary to describe such organisms as are killed by exposure to free molecular oxygen, as *strict* or *obligate* anaërobes, while the contrasting group, those which cannot function or survive without free molecular oxygen, are known as *strict* or *obligate* aërobes. Between these two extremes there apparently exists a continuous series of bacteria which possess in varying degrees the

¹ Vallery-Radot: *Life of Pasteur*, 1915, p. 99. (See also Pasteur, 1861, *C.r. de l'Acad. des Sci.*, 25 fév., 52, 344-347.)

ability of adapting themselves to either aërobic or anaërobic conditions. Collectively this intermediate group is known as facultative aërobes or facultative anaërobes.

Before leaving this subject it is necessary to call attention of a group of bacteria, spoken of as microaërophiles, or organisms living under reduced oxygen tension. To illustrate: the standard atmospheric pressure at 0° C. can be assumed to be 760 mm. of mercury. It is known that about 78 per cent by volume of the atmosphere is nitrogen and about 21 per cent by volume is oxygen. The molecular weights of these constituents are respectively about 28 and 32. With these data it is possible to calculate that a little over 23 per cent of the weight of the 760 mm. of mercury is accounted for by the weight of the oxygen in the atmosphere. This value is the normal oxygen tension. A lower value would represent a reduced oxygen tension, a condition favorable for the growth of certain bacteria, the microaërophiles. The gonococcus apparently is a member of this group and Rosenow believes that oxygen tension is a factor of importance in comparative virulence tests and in the demonstration of elective localization among the streptococci.

LIST OF PATHOGENIC (PARASITIC) ANAËROBES.

- Clostridium tetani.*
- C. botulinum.*
- C. welchii.*
- C. histolyticum.*
- Treponema pallidum.*
- T. pertenuis.*
- T. microdentium.*
- T. macrodentium.*
- T. mucosum.*
- Spironema duttoni.*
- S. vincenti.*
- S. refringens.*
- Fusiformis dentium.*
- Actinomyces* (some species or strains).

TECHNIC OF ANAËROBIC CULTIVATION.

The desideratum is of course the removal or elimination and exclusion of free molecular oxygen. Under natural conditions this is accomplished by the association of aërobes with the anaërobes. The aërobes exhaust the oxygen and thereby occasion anaërobic

conditions. Mixed cultures of aërobes and anaërobes can on this principle be artificially cultivated. But mixed cultures have a limited usefulness in bacteriology, so unless the aërobes die off in culture leaving the anaërobes as the sole survivors, little or no advantage is gained by this method. Even when the aërobes succumb, the anaërobes may consist of more than one species. Mixed cultures of anaërobes have proved very difficult to separate by ordinary methods of isolation; a fact which has led to much confusion and which only rather recently has been appreciated.

When anaërobic cultivations are attempted in jars it is advisable to use some sort of indicator for the presence or absence of oxygen. (1) One such indicator is a culture of a known anaërobe. A tube of medium is inoculated and put in the jar with the other tubes. If growth occurs it may be assumed that the removal of oxygen was satisfactory. One thing to be remembered in this connection is that many bacteria which are strict anaërobes when first isolated, become less exacting on prolonged artificial cultivation. (2) Another and very delicate indicator is afforded by an alkaline solution of pyrogallol. When used for this purpose the solutions of sodium hydroxid and of pyrogallol should not be mixed until one has reason to believe the anaërobiosis complete. If such a mixture becomes dark brown or black, the removal or exclusion of oxygen has not been satisfactory. (3) A very simple and reliable test for anaërobiosis is the decolorization of methylene blue in the presence of glucose in a slightly alkaline medium. Hall¹ has carefully studied the conditions of this reaction. It suffices to add a few drops of Loeffler's alkaline methylene blue, such as is commonly used for staining bacteria to a tube of neutral or alkaline glucose agar medium and put in boiling water. The medium loses its blue color completely. If then this tube be introduced into a jar and anaërobiosis be successfully induced, the medium will remain colorless. With the access of oxygen, the blue color will return.

1. Some anaërobes can be cultivated very simply by transferring some of the infected material into rather deep bouillon tubes at the bottom of which is a gram or two of finely chopped meat (muscle or liver). The meat is added before sterilization in the autoclave or if glucose has been added, in the Arnold. If enrichment is desired, $\frac{1}{10}$ to $\frac{1}{20}$ volume of sterile blood, whole or defibrinated or decalcified, or of blood serum, or of ascitic fluid, is added after sterilization. Such enriched medium should be incubated to be

¹ Journal Bacteriol., 1921, 6, 1.

sure of sterility before the actual inoculation. This method is useful to secure a primary culture but it does not ensure against mixed cultures. The process is somewhat simplified when one is seeking for endosporegenous anaërobes. In this case the fresh material is heated high enough to kill vegetative forms before transferring it to the medium.

2. A similar procedure is recommended by Rosenow.¹ Tall tubes of 0.2 per cent glucose broth, containing a few cubic centimeters of sheep's or calves' brain at the bottom, after sterilization afford anaërobic conditions in the deeper strata. Tetanus bacilli can be grown in this way. The introduction of a small piece of marble before sterilization will be of advantage in preventing the development of an inhibiting concentration of acids.

3. A very simple means of securing anaërobiosis is to boil the medium. The escaping bubbles of water vapor carry with them the absorbed or dissolved oxygen. If the medium be solid, as agar-agar, it should be rapidly solidified, inoculated with a number of stabs, and then at once covered with the contents of another agar tube, liquefied and cooled to about 50° C. In the case of liquid media or also in the case of solid media, sterile vaselin may be substituted for the agar as a seal. The seal of vaselin should be 3 or 4 cm. high. Liquid petrolatum once was used for this purpose but vaselin is preferable because it forms a seal far more impermeable to the atmospheric gases. The vaselin seal may be applied immediately after boiling the medium, and the inoculations may be made at any subsequent period directly through the seal. The inoculum can probably best be introduced by a capillary glass pipette. In such a case, however, care must be taken not to blow any air through the pipette into the medium.

When solid media are used the colonies can be seen developing along the lines of inoculation. Such colonies as are wanted for subculturing can be most directly approached by breaking the test-tube after sterilizing its surface with soap and water and mercuric chlorid (1 to 1000). The core of medium easily comes free and should be at once placed in a sterile Petri dish where the desired colonies can be "fished" out.

4. Pieces of freshly removed, living, sterile tissues at the bottom of tubes of liquid or semisolid media will secure anaërobiosis in their environment.

The functions performed by the fresh, living tissue are presum-

¹ Jour. Dent. Res., 1919, 1, 120.

ably several. During the continuance of its vitality, it utilizes oxygen which is thereby removed from the medium. During the autolysis of the tissue certain products, *e. g.*, amino-acids, are liberated which are of high nutritive value to the more perfectly adapted parasitic microorganisms. And finally during this autolysis, tissue enzymes are liberated, among which are peroxidases. There is evidence to believe that the toxic action of oxygen upon strict anaërobes is due to the inability of anaërobes to form peroxidases. If these are furnished by the autolyzing tissues, the growth of anaërobic bacteria is thereby favored.¹

Noguchi² has contributed much to the cultivation of anaërobic spirochetes; and all of his methods include the use of fresh, sterile tissue. The cultures are made in tall tubes, and the medium is to be covered with sterile vaselin to the depth of 3 or 4 cm.

Inasmuch as some spirochetes are such important oral bacteria and inasmuch as the securing of anaërobiosis is so bound up with other technical details, Noguchi's methods will be given in full at this place.

(a). For the cultivation of *Treponema microdentium*, *T. macrodentium* and *T. mucosum*, test-tubes with the dimensions, 20 cm. by 1.5 cm. are used. They are filled with 16 cc. of serum water (1 part sheep, horse, or rabbit serum plus 3 parts of distilled water). This is sterilized fractionally at 100° C. for fifteen minutes on each of three successive days. Then a small piece of freshly removed, sterile tissue (rabbit kidney or testis, preferable) is added. This medium is over-layered with sterile vaselin. After a primary growth has been obtained, transfers are made with a sterile, capillary pipette, for eventual isolation into serum agar (1 part sterile serum plus 3 parts sterile nutrient agar at ca. 42° C. poured into a tall tube at the bottom of which is a piece of sterile tissue). This is sealed before or after inoculation with sterile vaselin.

(b). A refinement of this method which gives a high percentage of successes in the cultivation of *T. pallidum*, is also well adapted for the oral spirochetes.

Methods. Construction of Culture Tube.—This consists of a test tube, 1.7 by 20 cm., whose bottom ends in a hollow projection made by fusing a short piece of strong glass tubing, 0.7 cm. bore, to the perforated bottom (Fig. 4, 1); a large test-tube, 2.5 by 15 cm. (Fig. 4, 2); and a perforated rubber cork, size 5 (Fig. 4, 3). These parts are assembled by connecting the two tubes by means of the rubber

¹ M'Leod and Gordon: Jour. Path. and Bacteriol., 1923, **26**, 332.

² Jour. Exper. Med., 1911, vol. **14**; 1912, vols. **15** and **16**.

cork (Fig. 4, 4). The upper tube is intended for solid and the lower for the fluid culture medium.

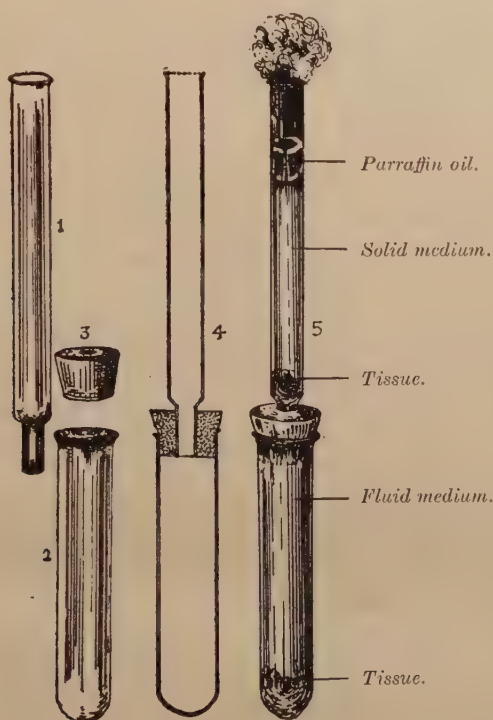


FIG. 4.—For description, see text, page 32.

Preparation for Cultivation.—The double tube just described is thoroughly cleaned and dried; the upper tube is then plugged with non-absorbent cotton. Just before use the whole is sterilized in an autoclave. In the meantime pieces of sterile, fresh rabbit kidney are prepared (one rabbit will usually furnish enough kidney substance for twelve such tubes). A slight alkaline agar (2 per cent) is also freshly prepared, autoclaved and kept in the liquid state.

Process of Cultivation.—(1) After the double tube cools, one or two pieces of sterile tissue is put directly in the lower tube. The double tube is then assembled and kept so throughout. Another piece of tissue, large enough not to fall through into the larger tube, is now put into the upper tube. (2) After the tissue has been placed in both tubes, the lower tube is filled with ascitic fluid or a mixture of ascitic fluid and bouillon. This is done by means of a

large sterile, bulb pipette whose tubular portion is drawn out so as to pass through the connecting tube with a margin of space that

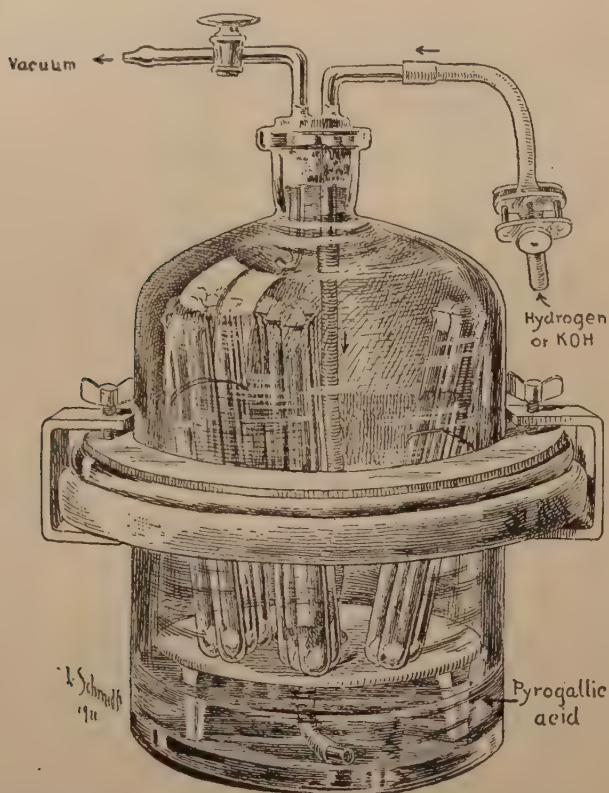


FIG. 5.—The apparatus in which three conditions can be combined. The inoculated test-tubes are placed inside of a jar which already contains a solution of pyrogalllic acid at the bottom. Hydrogen gas is first passed from the tube indicated through the pyrogalllic acid solution, for about ten minutes. After the air is driven off through the other tube (with stop-cock) the jar is sealed at both ends. The jar is then connected with a vacuum for about thirty minutes, in order to exhaust the air. When exhaustion has reached its maximum a concentrated solution of potassium hydrate is allowed to flow in from the tube indicated. Before the use of the potassium hydrate by passing hydrogen the solution is freed from oxygen as much as possible. The potassium hydrate solution is now mixed with the pyrogalllic acid solution in the jar. If there is any trace of oxygen inside the jar the mixture will quickly turn brownish or even dark brown, but if there is no oxygen it will remain almost colorless for at least many hours, when a light brown color may appear. The jar is now closed and once more hydrogen gas is passed through the mixture. Before putting the jar in the thermostat it is best to exhaust the air once more because this collapses the rubber tubing at one end, and a leak in the jar can easily be detected by the disappearance of the collapse (negative pressure) of this end. It has been found advisable to pass hydrogen gas through the inoculated culture media by means of a long, sterile capillary pipette for about five minutes before they are put in the anaerobic jar. (Noguchi.)

permits the air that is displaced by the fluid to escape. The lower tube must be filled so that there is no air bubble left inside the tube. (3) Inoculation; a "sufficient quantity" of well-growing culture is aspirated into a long capillary pipette. This is first introduced into the fluid of the lower tube into which some of its contents are forced. Then the remaining portion of the culture in the pipette is emptied into the upper tube just around the tissue. (4) The upper tube is filled with ca. 15 cc solid medium (1 part ascitic fluid plus 2 parts of slightly alkaline agar at about 42° C. in sterile flask), and then quickly after mixing poured into the upper tube. (5) Seal with sterile vaselin. The organism grows at first in the solid medium and then migrates into the fluid of the lower, larger tube.

(c). In Noguchi's earlier described cultivation of *T. pallidum*¹ the inoculation was made into tubes prepared as described above under (b). Then these tubes, sealed with vaselin, are to be placed in an anaërobic jar, in which the oxygen is removed according to the procedure outlined below.

5. Many methods of cultivating anaërobes depend exclusively or partially upon the fact that pyrogallol (pyrogallie acid), $C_6H_3(OH)_3$, in alkaline solutions absorbs molecular oxygen in large quantities. An aqueous solution of pyrogallol and one of sodium hydroxid are made up separately. Some procedure is followed which ensures the mixture of these two solutions after the inoculated tubes or plates have been so sealed as to prevent ingress of air. The resulting mixture after the absorption of oxygen becomes brown to black in color.

(a) A very simple application of this method may be used in connection with a single tube of medium. One cotton plug is moistened in the pyrogallol solution and pushed 2 or 3 cm. down in the tube. A few drops of sodium hydroxid solution are dropped in on top of this plug and a rubber stopper is at once tightly inserted.

(b) Buchner, who first used pyrogallol for anaërobiosis, used two test-tubes; the smaller being conveniently contained within the larger. The smaller contains the inoculated medium, and is supported several centimeters above the bottom of the larger tube. Into the latter is poured a few centimeters of sodium hydroxid solution into which are dropped several lumps of the pyrogallol. The mouth of the larger tube is at once sealed with a tightly fitting rubber stopper.

(c) Zinsser² has applied this principle to plate cultures. A Petri plate, somewhat deeper than that usually employed, is poured

¹ Jour. Exper. Med., 1911, 14, 99.

² Ibid., 1906, 8, 542.

and inoculated as usual. After solidification it is inverted. The smaller dish is carefully lifted out momentarily while a small quantity of pyrogallol solution is poured into the larger dish. The smaller dish is then replaced and into the crevice between the two dishes is poured some sodium hydroxid solution. The space between the two lids is then sealed with melted albolene or vaselin.

In addition to the three methods outlined above where sole reliance was placed upon the alkaline pyrogallol solution, there are numerous other methods, including those to be described, which often employ an alkaline pyrogallol solution to supplement some other measure for the removal of oxygen. In this connection the alkaline pyrogallol solution is very useful. If the other measure be effective the pyrogallol solution will remain colorless; if the other measure be more or less ineffective the incomplete removal or exclusion of oxygen will be indicated by the yellowing, browning or blackening of the pyrogallol solution which at the same time may satisfactorily take care of this excess of oxygen.

6. The mechanical displacement of the air by some inert gas, usually hydrogen, has long been in use for anaërobic studies. The inoculated plates or tubes are placed in a jar which can be hermetically sealed. Two tubes enter through the top of the jar; one of which goes to within 1 or 2 cm. of the bottom, the other only just passes through the top. This shorter tube is connected with an air pump for partial exhaustion. The longer tube is for the ingress of hydrogen from a Kipp generator or a tank. It once was customary to wash the hydrogen before passing into the anaërobic jar, but this is unnecessary. The jar is partially exhausted. Then the stop-cock on the exhaust tube is shut off and hydrogen run in as long as it will do so (the bubbling through a wash-bottle serves as a handy indicator). This alternate exhaustion and replacement by hydrogen is repeated a number of times. Then the stop-cocks on both tubes are shut off and the jar is placed in the incubator. (Fig. 5.)

This method is often supplemented by the use of pyrogallol. The bottom of the jar is covered with an aqueous solution of this substance and a small test-tube with a strong solution of sodium hydroxid is so propped against the plates or culture tubes in the jar that it can readily be overturned when desired. If the alkaline pyrogallol solution remains colorless, anaërobiosis has been secured. If any oxygen still remains in the jar its presence and absorption will be indicated by the yellowing or browning of the solution. Unless the color becomes very dark brown or blackish, the anaërobiosis obtaining will probably prove satisfactory.

7. It has long been known that finely divided platinum acts as a catalytic agent enormously accelerating the union of hydrogen and oxygen to form water. This principle was first applied by Laidlaw¹ to secure anaërobiosis. Fildes² presents some of the modifications of this method. The top of the jar is perforated for the ingress of hydrogen. Immediately under this perforation is attached a capsule, made of fine brass or copper gauze, which contains about 0.25 gm. of platinized or palladiumized asbestos. The gauze capsule is to cool the asbestos mass and prevent it from glowing red hot, and also to minimize the danger of explosion on the principle of the Davy lamp. In this method or in any other where oxygen is being combined with hydrogen, the danger of explosion always exists. Although no serious accidents have been reported, the care that is always necessary in working with hydrogen should be taken

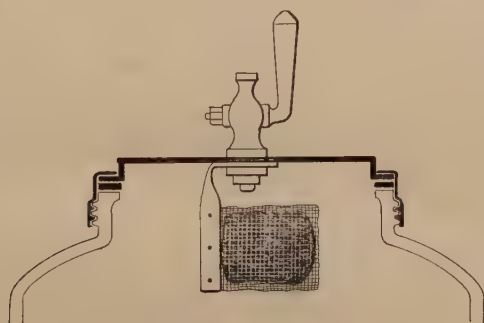


FIG. 6.—McIntosh and Fildes' anaërobic jar.

and it would be well if the entire jar during the operation of securing anaërobiosis were surrounded by some sort of muffler. A large cylindrical wire-basket wrapped and covered with several thickness of cheese-cloth will do.

The cultures are placed in the jar; the capsule is heated in a Bunsen burner, the lid of the jar is rapidly fixed in place and hydrogen is run in. As this combines with the oxygen, a negative pressure is created which tends to suck in more hydrogen. This continues until the oxygen is exhausted and no more hydrogen enters. When the apparatus has returned to room temperature, the stop-cock through which the hydrogen has entered, is shut off. The filling of the jar requires about fifteen minutes, during which time it requires no attention. (Fig. 6.)

¹ Brit. Med. Jour., 1915, i, 497.

² Special Report Series No. 12, National Health Insurance, Medical Research Committee, H. M. Stationery Office, London, 1917, pp. 66 *et seq.*

This method is easily applicable to single tubes or flasks, in which case the hydrogen is sent in through a tube perforating a rubber stopper, to the under surface of which is attached the capsule with the platinized or palladiumized asbestos.

8. A useful modification of the principle introduced by Laidlaw has been offered by Brown.¹ The initiation of the catalytic action

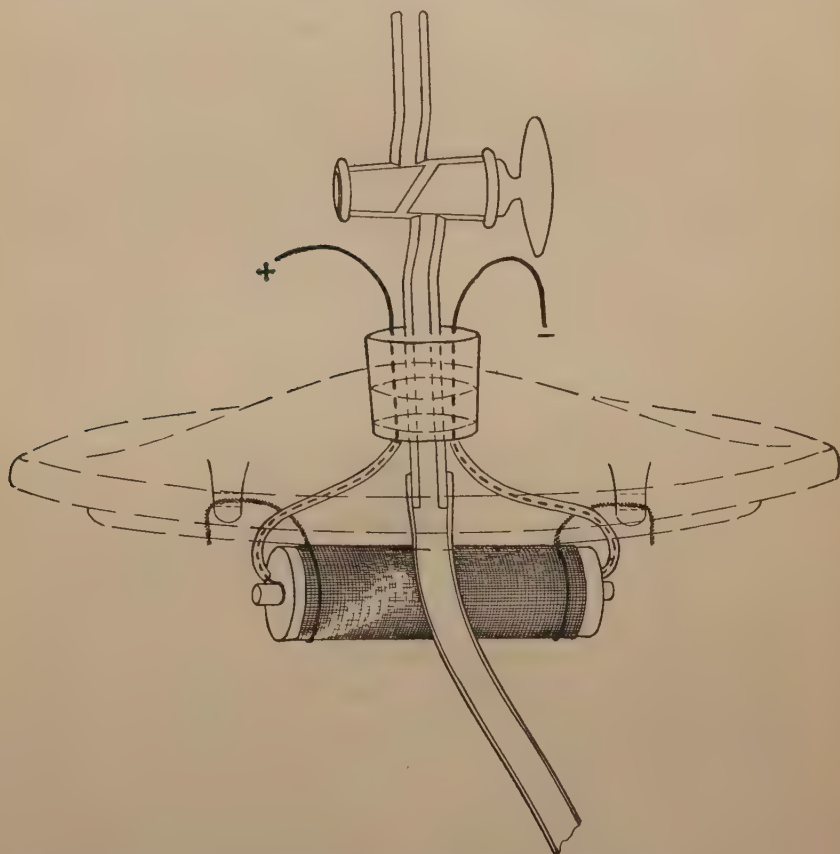


FIG. 7.—Coil enclosed by fine copper wire gauze and in position beneath the lid of the anaerobic jar. (Brown.)

which depends upon the heating of the platinized or palladiumized asbestos, is accomplished by a fine nichrome or platinum wire coil encircling the catalyzer. This coil is heated by the passage of an electric current through it. The construction of the essential part of the Brown apparatus is given by Figs. 7 and 8. It is advan-

¹ Jour. Exper. Med., 1921, 33, 677.

tageous to substitute asbestos disks for the rubber-stoppers (C_1 and C_2), because the heating sometimes suffices to burn the rubber. The coil and catalyzer are enclosed in fine-meshed copper wire screening, to minimize the danger of explosion. The gauge of the nichrome wire is B. & S. No. 28.

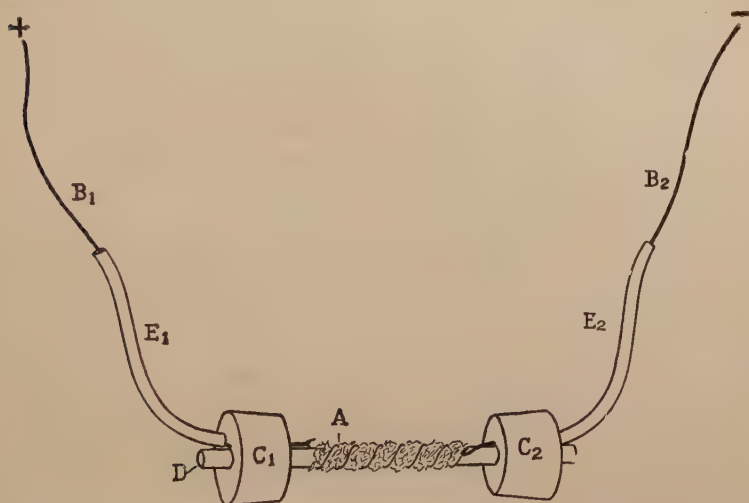


FIG. 8.—Construction of the catalyzer coil. *A*, the fine nichrome wire coiled about the platinized or palladinized asbestos; *B*₁ and *B*₂, large copper wires joined to the ends of the nichrome wire; *C*₁ and *C*₂, rubber stoppers; *D*, the core of glass tubing; *E*₁ and *E*₂, small rubber tubing serving as insulation. (Brown.)

The apparatus is manipulated as follows: The inoculated media are placed in the jar. The top is adjusted tightly. The perforating tube is attached to a suction pump and the jar is slightly exhausted. Then the electric current is sent through the coil until it is dull red and then reduced by means of a rheostat just until the red disappears. Then hydrogen is sent in through the perforating tube. As the hydrogen and oxygen combine the gas pressure within the jar is reduced and this tends to draw in more hydrogen, until all the oxygen is combined. When the inflow of hydrogen ceases the stop-cock is closed, the coil disconnected from the electric current and the jar incubated. In time the oxygen which was in solution in the media passes into the gases within the jar. The apparatus is again connected with the source of hydrogen and with the electric current in order to remove this oxygen. This possibility of repeatedly initiating catalysis at any time during incubation without reopening the jar, is the chief and very practical advantage of Brown's modification.

In reviewing the methods which have just been described, it is seen that at the present time in order to secure anaërobiosis, the removal of oxygen is accomplished in several ways, either used singly or in combination: (1) The activity of fresh, still living tissues; (2) the absorptive power of alkaline pyrogallol solutions; (3) mechanical displacement by some inert gas, usually hydrogen, and (4) the exhaustion of oxygen by chemical combination with hydrogen to form water under the catalytic influence of finely divided platinum or palladium.

ANAËROBIC BACTERIA OF THE MOUTH.

Treponema pallidum, the cause of syphilis, is found in the primary and secondary oral lesions of this disease. Further consideration of this organism will be given in the general discussion of syphilis.

Vincent's organisms, the *Spironema vincenti* and *Fusiformis dentium*, which are always present in Vincent's angina or stomatitis, ulcero-membranous stomatitis, trench mouth, and mercurial stomatitis, have been repeatedly cultivated.¹ Their characteristics and clinical significance will be described and discussed in the section on the special bacteriology of the mouth.

Little is known regarding the clinical bearings of the three treponemata isolated, as described above, by Noguchi: *T. microdentium*, *T. macrodentium*, and *T. mucosum*. They are certainly found in greatly increased numbers in certain stomatitides, gingivitis and periodontal affections.

Some species of *Actinomyces* or *Streptothrix* can only be isolated under anaërobic conditions. Wright² believed that an anërobic form was the principal agent in human and bovine cases of actinomycosis.

Luckett³ reported a fatal case of tetanus, in which the only discovered portal was the periapical region of a tooth. From this site was cultivated a typical strain of *Clostridium tetani*. A peculiar, postextraction case is described by Marañón and Velarde.⁴ They regard it as a case of larvate tetanus and the patient certainly improved remarkably after the administration of antitetanic serum. Such aberrant or atypical cases of tetanus are known. The tetanus bacillus has also been isolated from the mouth of a healthy child, as reported by Hall.⁵

¹ Weaver and Tunncliff: Jour. Infect. Dis., 1905, **2**, 446. Muehlens: Deutsch. med. Wehnschr., 1906, p. 797.

² Jour. Med. Res., 1904, **13**, 349.

³ Med. Rec., 1910, **17**, 319.

⁴ La Odontologia, Madrid, 1924, **33**, 513.

⁵ Meeting of the Society of American Bacteriologists, December, 1924.

Monier¹ was the first to make anaërobic studies of dental infections. His work was confined to 11 cases; 1 of cervical adenophlegmon, 1 of mandibular periosteitis with a fistula, 4 of osteoperiosteitis, all immediately followed death of the dental pulp, and 5 cases of acute pulpitis. Anaërobes were isolated from each of the 11, and in 2 cases only anaërobes were cultivated, *i. e.*, no aërobes developed on cultures from 2 of these 11 cases. The strict anaërobes found were *Bacillus ramosus* (in all 11 cases), *B. fragillus* (in 6 cases), *Micrococcus fœtidus* (in 1 case), the coccobacillus of Veillon and Morax (in 3 cases) an unidentifiable filamentous form (in 2 cases). Spirochetes were seen in smears but were not secured in cultures. Of the isolated anaërobes, all when injected into animals called forth fetid suppurations. This is interesting because the original material cultured from the human being in every case was of a very foul odor. Monier concludes on the basis of these pioneering researches that dental and periodontal lesions are not caused by a specific microbe but by a microbial association in which strict anaërobes play the principal role. Gangrene of the pulp and the sequent, more or less remote involvements have the same pathogenesis as do gangrenous and putrid processes in general; namely the activities of strict anaërobes.

Rodella² has found the *Clostridium putrificum* (Bienstock) in badly neglected mouths, in the calculus and food dëbris around teeth. Under similar conditions he also encountered one of the members of the group responsible for butyric fermentation, which he identifies as the *Granulobacillus saccharobutyricus immobilis liquefaciens* of Schattenfroh and Grasberger.³ A third anaërobe was the *Clostridium bifermentans* (Tissier and Martelly). Rodella isolated three strains of a gas-phlegmon bacillus which agrees with the description of an organism found by Schattenfroh and Grasberger.⁴

Baumgartner⁵ in concluding his study of diseased or putrid dental pulps from 20 cases, specifically states that such investigations must be carried on under anaërobic conditions. Contrary to what one might expect from this, most of his report is taken up with descriptions of microaërophiles and facultative anaërobes. Of strict anaërobes he found spirochetes only in smears. Fusiform bacilli he found in mixed culture. He is inclined to ascribe great

¹ Contribution a l'étude pathogénique des infections dentaires, Thèse de Paris, 1904.

² Arch. f. Hyg., 1905, **53**, 329.

³ Arch. f. Hyg., 1900, vol. **37**; idem, 1903, vol. **48**.

⁴ Loc. cit.

⁵ Oesterr.-ungar. Vrtljhrschrtf. f. Znhnlk., 1908, **24**, 352.

importance to these organisms (spirochetes and fusiform bacilli) in the gangrenous and liquefactive processes in dental pulps. As for other strict anaërobes, he only mentions that he was able to get in artificial culture some cocci and rod-like or filamentous forms.

Ozaki¹ describes two anaërobes isolated from the deposits on the teeth in apparently healthy mouths. The one is morphologically a fusiform bacillus which is non-pathogenic for mice, guinea-pigs or rabbits. The other is a minute staphylococcus, resembling the *S. parvulus* of Veillon and Zuber² and the *Micrococcus gazogenes alcalescens anaërobius* which was isolated by Lewkowicz³ from the mouth of a suckling. The minute staphylococcus of Ozaki was pathogenic for mice, guinea-pigs and rabbits. In a second contribution⁴ this author describes a small, Gram-positive, strictly anaërobic diplococcus, isolated from the deposits on the teeth of a normal mouth. It proved to be scarcely pathogenic for mice, guinea-pigs and rabbits. He decides that this organism is a close relative of, but not identical with, the following strict anaërobes: (1) *Micrococcus A* (Grigoroff); (2) *Staphylococcus parvulus* (Veillon and Zuber); (3) *M. gazogenes alcalescens anaërobius* (Lewkowicz); (4) *Staphylococcus minimus* (Gioelli); (5) *M. fætidus*. (Veillon); (6) The staphylococcus described by Ozaki in his first (1912) contribution, and (7) *Staphylococcus aërogenes* (Schottmüller).

Idman⁵ published a bacteriological investigation directed particularly at the anaërobes found in the periosteal abscesses associated with purulent and gangrenous pulpitis. He studied 8 cases intensively, both aërobically and anaërobically. Each of the 8 cases yielded aërobic (facultative) and anaërobic bacteria, usually with the latter group predominating. The table on p. 43, adapted from one in Idman's work in which he summarized some of Monier's observations⁶ and his own, shows that anaërobic bacteria in oral conditions should not be ignored.

Gilmer and Moody⁷ cultured alveolar abscesses and infected root canals both aërobically and anaërobically. A fusiform bacillus was not infrequently encountered, sometimes in practically pure culture. They also noted in old anaërobic cultures from three different abscesses a black chromogenic organism, possibly that described as *Bacterium melaninogenicum* by Oliver and Wherry in 1921.

¹ Centralbl. f. Bakteriöl., 1912, **62**, 76.

² Arch. de méd. exper. et d'anat. path., 1898, **10**, 517. ³ Ibid., 1901, **13**, 633.

⁴ Centralbl. f. Bakteriöl., 1915, **76**, 118.

⁵ Arb. a. d. path. Inst. d. Univ. Helsingfors, Finland, 1913, **1**, 191.

⁶ Thèse de Paris, 1904.

⁷ Jour. Am. Med. Assn., 1914, **63**, 2023.

Obligate anaërobes.	Cases.												
	Monier.					Idman.							
	VI.	VII.	IX.	X.	XI.	A.	B.	C.	D.	E.	F.	G.	H.
Streptococci	..	..	..	+	..	+	..	+	+	..	..	+	
Staphylococcus parvulus (Veillon and Zuber)	..	..	..	..	..	..	..	..	+	..	+		
Staphylococcus jugano	..	..	..	..	..	..	..	..	..	..	+		
B. ramosus (Veillon and Zuber)	+	+	+	+	+	+	+	+	+	+	+	+	+
B. thetoides (Rist and Guillemot)	..	..	..	..	..	..	..	+	+	..	+		
B. fragilis (Veillon and Zuber)	..	+	+	+	..	..	..	..	..	..	..		
B. perfringens (Veillon and Zuber)	..	+	+	+	..	..	..	+	+	..	..		
B. fusiformis	..	..	..	..	..	..	..	..	..	..	+		
B. bifidus communis (Tissier)	..	..	..	..	..	..	..	..	+	..	..		
Other rods	..	..	+	+	..	+	..	..	+	..	..		

Kritchevsky and Seguin¹ remark that among other anaërobes obtained from the depths of pyorrhea pockets *M. parvulus* and *M. fœtidus* of Veillon are often encountered. Some strains of these organisms have proved to be pyrogenic for laboratory animals.

Kliger² in his studies on oral bacteria found the anaërobe, *Bacillus putrificus*. By virtue of its powerful proteolytic properties, he ascribes to it a close relation to the process of pulp decay.

Sommer³ has made anaërobic studies on 12 cases of pulp gangrene. One hundred and nine anaërobic cultures were secured, showing the following distribution: *Bacillus fusiformis*, 67 times; *Granulobacillus* sp., 34 times; and *Bacillus perfringens*, 5 times. Spirochetes were seen in smears but their cultivation was never successful. The fusiform bacillus probably was identical with that isolated by Ozaki (1912) and Idman (1913). The comparatively few times in which *B. ramosus* was found contrasts with the almost constant occurrence of this organism in the dental lesions of Monier (1904) and Idman (1913). None of the cultures obtained by Sommer exhibited any real pathogenicity. His view apparently is that after the death of the pulp, the anaërobic types begin to gain the upper hand and predominate during the phase of pulp dissolution which is known as gangrene.

Mendel⁴ mentions four anaërobes isolated from oral lesions: (a) *Staphylococcus parvulus*, Veillon; (b) a small "Vibron du type *Repaci B*;" (c) an unidentified, Gram-negative, sporogenous bacillus,

¹ Dent. Surg., 1924, **21**, 297.

² Jour. Allied Dent. Soc., 1915, **10**, 141, 282, 445.

³ Deutsche Monatschrift fuer Zahnheilkunde, 1915, **33**, 297.

⁴ Compt. rend. Soc. de biol., 1917, **80**, 962.

and (d) a Gram-positive bacillus very similar to the diphtheroid of Jungano. The first three forms were isolated from pyorrhetic pockets and the fourth from a periapical infection.

Oliver and Wherry² describe two obligate anaërobes which may occur in the mouth. The first is named by them *Bacterium melaninogenicum*. They have isolated it from various human sources and suggest it may be the organism appearing as black colonies on anaërobic blood-agar cultures from carious teeth. The second organism is named *Micrococcus minutissimus* and was isolated from the aphthous ulcers of the gingival and buccal mucosa of a case of postpoliomyelitic paralysis. It proved to be non-pathogenic toward the white mouse, guinea-pig or rabbit.

Summary.—In summarizing the reports of Monier, Rodella, Baumgartner, Ozaki, Idman, Gilmer and Moody, Kliger, Sommer, and Oliver and Wherry, it appears that Ozaki, and Oliver and Wherry have described oral anaërobes of little or no clinical significance. On the other hand it becomes obvious from the work of Monier, Rodella, Idman, Kliger and Sommer that anaërobes are very frequently or always present in gangrene of the dental pulp, which are also known to occur in other gangrenous processes, and that such anaërobes are possessed of properties which would seem to account for the clinical facts of pulp dissolution. Under similar or perhaps more acute conditions, fusiform bacilli, sometimes associated with spirochetes, have been found, *e. g.*, by Baumgartner, Ozaki, Idman, Gilmer and Moody, and Sommer.

The technical difficulties inherent in anaërobic cultivation account for the relatively few times it has been applied to oral infections. The review and summary given above should emphasize the fact that this field is still largely a *terra incognita*.

¹ Jour. Infect. Dis., 1921, 28, 341.

CHAPTER III.

CHEMICAL DISINFECTANTS.

THE HARMFUL ACTION OF CHEMICALS ON BACTERIA. DISINFECTANTS, ANTISEPTICS, GERMICIDES.

It has been customary to attempt to make distinctions between disinfectants, antiseptics and germicides. A disinfectant is said to destroy infectious agents, irrespective of its action or inaction upon non-infectious microbes. An antiseptic inhibits but does not necessarily kill microorganisms. A germicide kills microorganisms and their spores. These distinctions possess slight or no usefulness. They merely represent differences of degree depending upon the concentration of the chemical substance employed.

The mechanism by which the vitality of the cell is destroyed is still largely unknown. The equilibrium of the intracellular processes essential to "life" is irrecoverably disturbed. This might be done in a number of different ways; including desiccation, coagulation of the proteinogenous constituents of protoplasm by alteration of physical state or by chemical combination, changing the permeability of the cell wall, changing the rates of cellular oxidation or reduction, union of the "disinfectant" with some of the constituents of protoplasm with the formation of a relatively stable compound. It is also conceivable that certain "disinfectants" exert their harmful influence upon the bacterial cell, as catalytic agents; modifying rates of endocellular reactions, possibly interfering with the detoxication of metabolites or unduly accelerating the production of toxic metabolites. Enzymes are often sensitive as to the environment or medium in which they act, and "disinfectants" may disturb enzyme activity. Most of this paragraph, it must be admitted, consists of surmises and it must not be forgotten that our knowledge of the ultimate mechanism by which chemicals injure and inactivate living protoplasm is woefully lacking.

Factors in Chemical Sterilization.—From a more practical and more empiric standpoint we must content ourselves with an enumeration and discussion of some of the more obvious factors influencing the "disinfectant" action of chemicals in solution,

1. *Nature of Microörganism.*—Different species of bacteria may be differently affected. J. W. Churchman¹ found that gentian violet present in a dilution of 1 to 1,000,000 in culture media prevented the growth of a large majority of Gram-positive bacteria. On the other hand a large majority of Gram-negative bacteria are uninfluenced by the presence of the dye in this dilution, and often will grow in one of even 1 to 1000. This parallelism between Gram-positiveness and sensitivity to gentian violet presumably is due to the presence of some constituent within the bacterial cell, which is absent in those organisms which are Gram-negative and insensitive to gentian violet; although from a recent study Churchman² believes that Gram-positiveness and sensitivity are not due to one and the same constituent or factor. The simple aniline derivatives, as well as the dyes, are more toxic for the Gram-positive than the Gram-negative bacteria. Of the former, *B. subtilis* is more sensitive to the dyes than *Staphylococcus aureus*, while the reverse is true in the case of aniline compounds.³ The following table from Gay and Beckwith⁴ shows the difference between Gram-positive and Gram-negative bacteria in sensitivity to methylene blue (M. P.) in peptone solution. The dilutions are the highest having bactericidal effect under the conditions of the experiment.

<i>A. Gram-positive.</i>		
<i>B. anthracis</i>		1 to 100,000
<i>B. hoffmanni</i>		1 to 100,000
<i>Staphylococcus aureus</i>		1 to 100,000
<i>Streptococcus pyogenes</i>		1 to 80,000
<i>B. smegmatis</i>		1 to 1,000
<i>B. subtilis</i>		1 to 1,000
<i>B. Gram-negative.</i>		
<i>B. prodigiosus</i>		1 to 10,000
<i>B. mucosus capsulatus</i>		1 to 1,000
<i>B. proteus</i>		1 to 1,000
<i>B. pyocyaneus</i>		1 to 1,000
<i>B. typhosus</i>		1 to 500
<i>Saccharomyces cerevisiæ</i>		1 to 200

This differential sensitivity between Gram-positive and Gram-negative bacteria has been frequently noted.⁵ Bail⁶ found Gram-positive bacteria more susceptible to the oligodynamic action of metals than the Gram-negative. *B. coli* is about 10 per cent more

¹ Jour. Exper. Med., 1912, **16**, 221.

² Proc. Soc. Exper. Biol. and Med., 1920-1921, **18**, 17.

³ Kliger: Jour. Exper. Med., 1918, **27**, 463.

⁴ Am. Jour. Hyg., 1923, **2**, 473.

⁵ Eisenberg, R.: Centralbl. f. Bakteriöl., 1913, **71**, 420. Kolmer, Woody and Yagle; Jour. Inf. Dis., 1920, **26**, 179.

⁶ Wien. klin. Wchnschr., 1919, No. 19,

resistant than *B. typhosus* in the Rideal-Walker test.¹ Kligler² found a most marked specific selective effect manifested by the triphenylmethane dyes. *B. aërogenes* and *B. typhosus* possess a higher resistance to these substances than *B. coli* or *B. dysenteriae*. Lewis³ enumerates a great variety of substances possessing a marked capacity to restrain the growth of the tubercle bacillus which were relatively much less active against *B. typhosus*. The general disinfectant action of the dyes, as represented by the results with *B. typhosus*, bore no simple relation to their inhibiting power on the growth of *B. tuberculosis*.

2. *Variations in the Vital Resistance of the Same Species*.—This includes not only different regular stages in the life cycle of the microorganism, but also slighter, transient, inexplicable irregularities. The bacterial endospore is far more resistant than the vegetative stage of the same organism. A 10 per cent solution of creolin kills anthrax bacilli in ten to twenty minutes, but is unable to effect the death of anthrax spores. These maintain their vitality in even a 60 per cent solution.⁴

3. *The Nature of the Disinfectant: its Chemical Composition and Molecular Structure*.—The classical study in this field is that of Kroenig and Paul.⁵ In 1881, J. Blake⁶ found that in isomorphous groups the toxicity increases with the atomic weight, while a variation in the anions of metallic salts has but little effect on the toxicity. In the case of the non-metals this relationship does not hold; thus in the case of the halogens, the toxicity appears to increase in the order I₂, Br₂, Cl₂, and for the sodium halids NaCl, NaBr, NaI, and NaF.⁷

Kroenig and Paul⁸ first gave considerable attention to the mechanism of action of metallic salts. The disinfectant action of these in solution is dependent not only upon the concentration of the metal present in the solution, but is also dependent upon specific peculiarities of the salt and of the solvent. Rideal and Rideal point out (p. 190) that the valency of the metallic ion is an important factor in germicidal activity, thus trivalent ions are more potent than bivalent, bivalent than monovalent (univalent) ions.

Again, unsaturation augments the germicidal power; thus trivalent arsenic is a much more efficient spirillicide than pentavalent,

¹ Rideal and Rideal: Chemical Disinfection and Sterilization, London, 1921, p. 290.

² Op. cit.

³ Jour. Exper. Med., 1917, 25, 441.

⁴ Jordan: General Bacteriology, Philadelphia, 6th ed., 1918, p. 246.

⁵ Ztschr. f. Hyg. u. Infek., 1897, 25, 1.

⁶ Proc. Roy. Soc., B. London, 1881, 14, 299.

⁷ Rideal and Rideal: Op. cit., p. 190.

⁸ Op. cit.,

as are the stibnites as compared with the stibnates. The protocols of Kroenig and Paul indicate that solutions of metallic salts in which the metallic constituent of a complex ion and accordingly the concentration of the metal ions is very small, exhibit extraordinarily weak disinfectant powers. The action of a metallic salt depends not only upon the specific action of the metal ion, but also upon that of the anion, and of the undissociated fraction. The disinfectant action of aqueous solutions of mercuric chlorid is lowered by the addition of metallic halides and of hydrochloric acid. Apparently this decrease in disinfectant power depends upon the lessening of electrolytic association. The disinfectant action of aqueous solutions of mercuric nitrate, mercuric sulphate, mercuric acetate, is materially heightened by the addition of sodium chlorid.

Acids exert disinfectant action in general in relation to their degree of electrolytic dissociation, *i. e.*, corresponding to the concentration of hydrogen ions contained in the solution. The anions and the undissociated molecules of hydrofluoric acid, nitric acid and trichloroacetic acid exhibit a specific toxic action. This diminished with increasing dilution in contrast to the toxicity of hydrogen ions. As Rideal and Rideal emphasize (p. 192) in completely ionized dilute acids there is scarcely any selective effect due to the anion, but with stronger solutions the germicidal power is by no means proportional to the hydrogen-ion concentration; marked anion effects are noted in the case of HF, HNO₃, HCl, and C₂H₂O₄, which are all abnormally strong germicides. Sierakowski and Milejkowska¹ found that the lethal concentrations of H and OH ions varied with different species, but were quite constant for the same species. *B. coli* was killed at pH 3.8 while *B. typhosus* and the dysentery species were killed at pH 4.2 to 4.6. *V. cholerae* was killed at pH 5.3. The lethal OH concentration for this group ranged between pH 9.6 to 10.45. *B. diphtheriae* was killed at pH 4.9 and pH 10.00 while the bacillus of Hoffman was killed at pH 5.3 to 11.31. The staphylococci were killed by concentrations of pH 3.9 to 4.6 and pH 11.9 to 12.2. The meningococcus proved to be the most sensitive, being killed by pH 6.9 and pH 9.0. The Gram-positive spore-formers were not killed by the lowest and highest concentrations used, pH 1.7 and pH 13.0.

The bases, the hydroxids of potassium, sodium, lithium, ammonium, disinfect in proportion to their degrees of dissociation, *i. e.*, corresponding to the concentration of the hydroxyl ions contained in the solution. The activity of the hydroxyl ion is some five times

¹ Compt. rend. Soc. de biol., 1924, **90**, 714.

less than that of the hydrogen ion. The strong alkalies KOH, NaOH, LOH, are practically equally effective.¹ Hydrogen ions are more toxic for the spores of *B. anthracis* and in a higher degree for *M. aureus* than hydroxyl ions in the same concentration.

The disinfectant action of the halogens, chlorin, bromin, and iodine, decreases with increasing atomic weights.

Oxidizing agents, *e. g.*, nitric acid, bichromates, chlorates, persulphates, and permanganates act according to their position in the series established for oxidizing agents on the basis of their electrical behavior. Chlorine does not fit into this series but exerts a very strong specific action. The disinfectant action of different oxidizing agents is significantly increased by the addition of the halogen acids (*e. g.*, potassium permanganate with hydrochloric acid).

While Kroenig and Paul practically limited their attention to the compounds of inorganic chemistry, Kliger² studied a limited group of organic compounds. His principal findings, pertinent to the question of the influence of chemical composition and structure of a disinfectant are summarized below. The germicidal action of the compounds (aniline and some of its derivatives and the triphenylmethane dyes) is a function of the benzene nucleus, the added elements or radicals, their number, and, in the case of the dyes, probably the quinoid structure of the nucleus.

An increase in the number of alkyl radicals increases the antiseptic power. Methyl green is an interesting exception to this rule, for the change of one of the nitrogens to the quarternary salt is accompanied by an almost complete loss in inhibitive action.

The antiseptic power is enhanced to a greater extent by an ethyl than by a methyl group, and the second alkyl produces a proportionately greater increase than the first. It appears that the relative position of the introduced group may be a factor in determining the relative improvement in the effectiveness of the compound.

The introduction of a methyl group in the nucleus consistently enhances the inhibitive action of the compound and its alkyl derivatives. This is evident from a comparison of the action of aniline and its derivatives with that of toluidine and its corresponding derivatives.

Chemotherapy.—The importance of chemical composition and molecular structure is fully appreciated in the study of chemotherapy. One of its problems is to find a substance which exerts a specific destructive action upon the microorganism in concentrations causing a minimum of disturbance in the macroorganism. Empirically the

¹ Rideal and Rideal: p. 192.

² *Op. cit.*

specific curative action of mercury and iodids in syphilis, of quinin in malaria, and of ipecac in amœbic dysentery has long been known. The most conspicuous achievement of the scientific development of this field was the discovery of the spirocheticidal action of salvarsan and neosalvarsan by Ehrlich. As early probably as 1910, Ehrlich himself suggested the use of salvarsan in the treatment of oral spirochetal infections.¹ As another example of chemotherapy, possibly with a dental application, may be mentioned the work of Kolmer and others on the action of ethylhydrocuprein upon pneumococci.² Bruhn³ reported that certain chinin derivatives (*e. g.* hydrochinin, optochin, eukupin, and vuzin) actually secured, in contrast to methods hitherto used, more or less markedly favorable results in root-canal treatment, purulent periodontitis, stomatitides, "pyorrhea alveolaris," etc. Bruhn's experience has been that most infectious processes can be treated with these chemotherapeutic agents more easily, more certainly, and with less pain than has hitherto been possible.

4. *The Solvent for the Disinfectant.*—The solvent must not combine chemically with the disinfectant to form an inert substance. If a new compound be formed, which however is effective, that new compound becomes itself the subject of the test and not the original chemical. Other things being equal, that solvent is most efficacious which permits the highest degree of dissociation of the disinfectant (if this be an electrolyte); *i. e.*, which permits the highest degree of ionic activity.

Disinfectants are far less effective in anhydrous liquids than in water. The influence of the solvent upon the germicidal action of a solute has long been known. Lister at one time or another for various purposes tried phenol dissolved in olive oil, linseed oil, beeswax, paraffin cerate, "spirits of wine," etc. He recognized that in these vehicles carbolic acid was less irritating to living cells than in water; and reference is made to this in his earliest classical paper on the antiseptic system.⁴ In some remarks introduced parenthetically at a later day into a pamphlet published in 1870, he explicitly states, after speaking of solutions of phenol in glycerin or alcohol, that "to kill the putrefactive (pathogenic) organisms . . . no vehicle seems better for the acid (phenol) than simple water."

¹ Sitzung d. Aerzte-vereins Frankfurt a. M.

² Jour. Am. Med. Assn., 1918, 70, 14.

³ Ergb. d. ges. Zahnk., 1920, 6, 154.

⁴ Lister, Joseph: On a New Method of Treating Compound Fracture, Abscess, etc., Lancet, 1867, i, 326.

Robert Koch² in his very extensive survey of the field of chemical sterilization had his attention directed repeatedly to the influence of the solvent. He observed that 5 per cent of valerianic, palmitic, stearic, or oleic acid in ether failed to kill anthrax spores in five days; that 5 per cent xylol in alcohol was ineffective in ninety days; 5 per cent thymol or salicylic acid in alcohol, ineffective in fifteen days; 2 per cent salicylic acid in oil, ineffective in eighty days; 5 per cent *oleum animale* in oil, ineffective in twelve days; and 5 per cent *oleum menthi piperita* in alcohol, ineffective in twelve days. The time periods specified were apparently quite accidentally chosen and are certainly not to be taken to mean the maximum limit of viability. For all that the test shows the periods of exposure of the anthrax spores to these solutions might have been indefinitely prolonged without germicidal action.

The following data from Koch's work serve to contrast the action of phenol in aqueous and in anhydrous solutions. A 3 per cent aqueous solution of phenol killed anthrax spores in seven days, but not in five; a 4 per cent solution killed in three days, but not in two; a 5 per cent solution killed in two days, but not in one. On the other hand, a 5 per cent solution of phenol in oil or in alcohol exerted no apparent deleterious action upon anthrax spores even after one hundred and ten days of contact. Anthrax bacilli, in the more sensitive vegetative, not in the resistant endospore stage, survived four days in a 1 or 5 per cent solution of phenol in oil but had succumbed by the sixth day. *Identical results in this particular were obtained with pure olive oil.*

In these studies Koch tested the germicidal action of carbon-disulphid, ether, chloroform, benzol, petroleum ether and turpentine oil, with no notable results except in the case of ether and oil of turpentine. Anthrax spores immersed in ether survived at least eight days but had succumbed by the thirteenth day. Turpentine oil killed anthrax spores in five days, but not in one. Koch implies that possibly the ozone-carrying property of ether and oil of turpentine may be responsible for the not inconsiderable disinfectant power of these liquids in comparison with the other substances tested. Although water to which a few drops of oil of turpentine had been added did not kill anthrax spores in ten days, Koch did not give up hope "dass sich das Terpentin-Oel in irgend einer Form, vielleicht in Combination mit trockner oder feuchter Hitze als Desinfektionsmittel verwerthen lassen wird" (that oil of turpentine will in some

² Ueber Desinfektion, Mitt. a. d. k. Gsndhtsamte., 1881, I.

form or other, perhaps in combination with dry or moist heat, prove to be of value as a disinfectant agent).

These early observations of Lister and Koch that the choice of the solvent is not immaterial in disinfection have been repeatedly confirmed. Kroenig and Paul¹ report a number of findings bearing upon the influence of solvent. Substances dissolved in absolute ethyl alcohol, in methyl alcohol or ethyl ether are almost without action upon spores of *B. anthracis*. Similarly every addition of ethyl or methyl alcohol to an aqueous solution of phenol or of formic aldehyde decreases its disinfectant action. On the other hand, the addition of definite volumes of ethyl alcohol, methyl alcohol and acetone to aqueous solutions of silver nitrate or mercuric chlorid will significantly increase their disinfectant action.

Among the factors which suggest themselves as worthy of consideration in an attempt to explain this generally recognized fact are: (1) The state of the solute in the solvent; (2) the relative solubility of the solute in the solvent on the one hand and in the bacterial protoplasm on the other, *i. e.*, the partition coefficient; (3) the diffusibility of the solute from the solvent into the aqueous solution within the cell (the bacterial protoplasm), and (4) effective *chemical* contact of the solute (or solvent) with the bacterial cell, *i. e.*, does the solvent "wet" the cell?

It was suggested at least as early as Wolffhugel and v. Knorre² that possibly the unsatisfactoriness of oily solutions of disinfectants lay in part in the tendency of such solutions not to "wet" the object on or in which the bacteria were. The factors influencing this "contact" may be: (1) The miscibility of the solvent with aqueous solutions (protoplasm) or with constituents of the bacterial cell wall; (2) the absorption, positive or negative, of the solute at the surface of the solvent; (3) the surface tension of the solvent which is of course influenced by (2). It is a matter of common observation that it is difficult to "wet" certain objects which are thoroughly dry, *i. e.*, it is difficult for the liquid to come in actual contact with the object.

5. *Concentration of the Disinfectant.*—It is not in all cases true that the higher the concentration, the higher the disinfectant action. This is explicable on the ground of dissociability, as indicated in No. 4. The higher the concentration, the fewer are the molecules which are at any given moment dissociated. Germicidal

¹ Op. cit.

² Zu der verschiedenen Wirksamkeit von Carboloeel und Carbolwasser, Mitt. a. d. k. Gsndhtsamte, 1881, 1, 352.

power is in direct proportion to the degree of ionization, *e. g.*, a 1 to 500 aqueous solution of HgCl_2 is much less than twice as active as a 1 to 1000 solution. Similarly, of the mercuric halids, in equivalent solutions, the chlorid is more active than the bromid, and four times more active than the cyanid, which is almost un-ionized.¹ In brief as Kroenig and Paul² found, the effectiveness of the halids of mercury is proportional to their degree of dissociation. Rideal and Rideal³ further point out that the antiseptic power of the aliphatic acids runs parallel with their *dissociation constant*; both decreasing with increasing molecular weight.

The maximum disinfectant action of ethyl alcohol is at 70 per cent. Although this is not a question of dissociation, it is one of concentration.

6. *The Amount of the Disinfectant Added to the Bacterial Culture, i. e., the Proportion of Culture to Disinfectant.* The following table⁴ illustrates the effect of varying the proportion between the disinfectant and the number of bacteria. The medium was infusion broth and the disinfectant was acriflavine. The figures indicate the highest bactericidal dilution.

	In peptone.
For 10 cc culture <i>Streptococcus hemolyticus</i>	1 to 8,000
For 1 cc culture <i>Streptococcus hemolyticus</i>	1 to 64,000
For 0.1 cc culture <i>Streptococcus hemolyticus</i>	1 to 128,000
For 0.01 cc culture <i>Streptococcus hemolyticus</i>	1 to 256,000

It is obvious that the greater the number of bacteria, the higher must be the concentration of the disinfectant to effect sterilization.

7. *Temperature at Which the Test is Performed.*—The higher the temperature, the more rapid is the disinfectant action. It is obvious that the temperatures employed must not be incompatible with the life of the microorganism. If we use lethal temperature, the problem is at once complicated. (See Fig. 9.)

8. *Time.*—Time during which the microorganism is left in contact with the disinfectant.

9. *Nature of the Medium in Which the Microorganism is Suspended; Colloidal or Crystalloidal, Organic or Inorganic, its Hydrogen-ion Concentration, etc.*—J. H. Wright⁵ on the basis of careful study of the composition of culture media concludes that there is a marked relationship between the hydrogen-ion concentration of the culture medium and the resistance of the test organism to the action of disinfectants.

¹ Rideal and Rideal: Op. cit., p. 208.

² Op. cit.

⁴ Gay and Beckwith: Op. cit., p. 474.

³ Op. cit., p. 213.

⁵ Jour. Bacteriol., 1917, 2, 315.

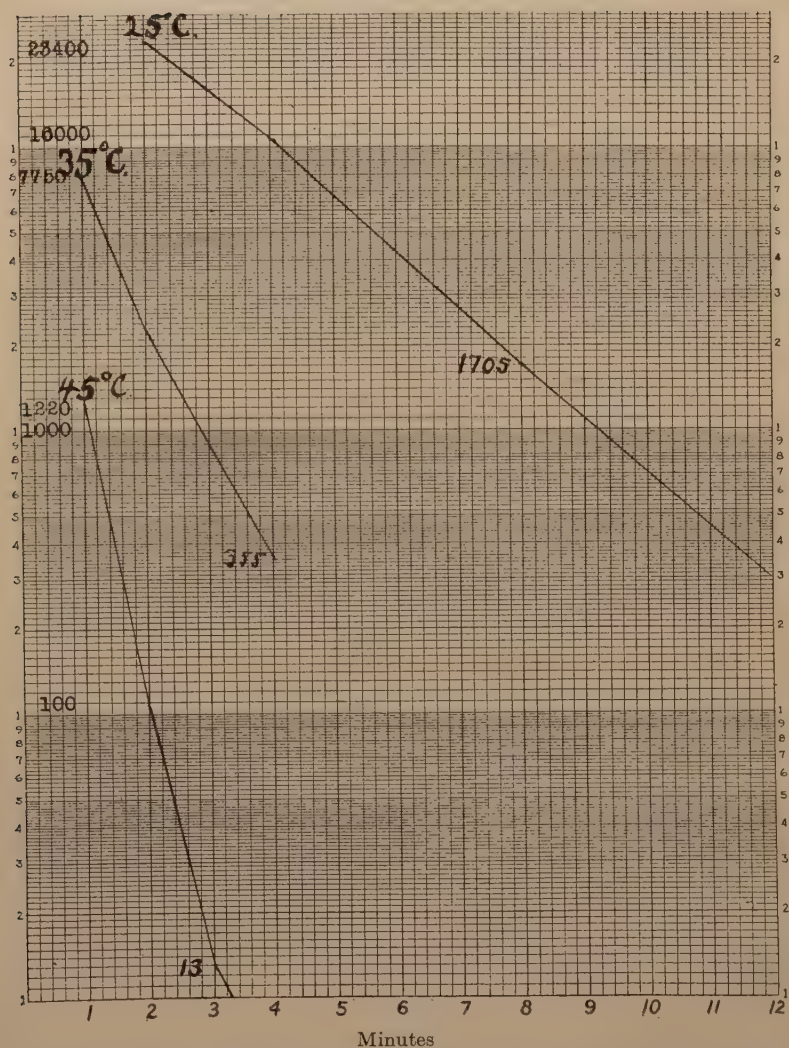


FIG. 9.—Influence of temperature on chemical sterilization. Constructed on data given in Table 15 of Madsen and Nyman (*Ztschr. f. Hyg.*, 1907, **57**, 400). The chemical employed was HgCl_2 in aqueous solution. The initial number of bacteria (*B. anthracis* spore) was the same in the three tests. The numbers at the left indicate the number of bacteria surviving after the exposures indicated on the base line. These graphs are given on "semi-log" paper and show that the rate at which the bacteria are destroyed is the greater for the higher temperature. Control tests in which HgCl_2 was not used showed that the temperatures in themselves did not influence the number of bacteria.

The following results¹ illustrate the importance of hydrogen-ion concentration. In nutrient broth at a pH 6.2 *B. aërogenes* is able to grow in the presence of caffeine (1 to 100); while no growth occurs at pH 7.4.

Under the same conditions *B. typhosus* shows no growth in the presence of dibromobeta-naphthol (1 to 12,500) at pH 7.4 but growth occurs at pH 8.2. When the pH is 6.2, *B. dysenteriae* (Flexner), *B. dysenteriae* (Shiga), and *B. typhosus* show no growth in the presence of hexamethylenetetramine (quaternary salt of p-chloroacetylaminoleukomalachite green) (1 to 10,000); but in each case growth occurs at pH 7.4. *B. coli communior* is inhibited by hexamethylenetetramine (quaternary salt of chloroacetyethylamine (1 to 10,000) when the pH is 6.2 but there is no inhibition of growth at pH 7.4.²

Kroenig and Paul³ reported that the disinfectant action of metallic salts is in general less in bouillon, gelatin, body fluids or in watery solutions to which the above or similar liquids have been added, than in purely aqueous solutions. Apparently this decrease in the disinfectant action rests upon a diminution of the concentration of metal ions. Another instance of the same nature is afforded by Kliger⁴ who found that the higher the concentration of organic nitrogenous compounds (peptone) in the medium, the lower is the effective concentration of the dye.

In any series of tests on chemical sterilization consideration must be given to these factors. According to the point of view of the investigator one or more of them must be variable while the rest are maintained as constants.

Antisepsis and Asepsis in Dentistry.—Inasmuch as the mouth always contains bacteria and an infectious factor is prominent either as a primary or secondary phenomenon in most of the conditions the dentist is called to meet, bacterial-inhibiting or bacterial-killing chemicals have constituted an important part of the dental materia medica. This is not the place to consider these and it will suffice to say that the present tendency is to avoid the use of strong or irritating disinfectants upon living tissues. This applies to mouth-washes as well as to the solutions used in gingivitis, "pyorrhea alveolaris" and periapical infection. The rule is almost generally valid that what will injure the bacterial cell will do at least as much damage to the human cell. This tendency has been hastened by the

¹ Kliger: Op. cit., 1918.

² Browning, Gilbransen and Kennaway: Jour. Path. and Bacteriol., 1919, 23, 106.

³ Op. cit.

⁴ Op. cit.

experiences of the World War in which the chlorin group of disinfectants, such as Dakin's solution and dichloramin-T, proved so effective, largely because the human tissues tolerated these substances so well.

Oligodynamic Action.—Some of the materials used in operative or prosthetic practice exert a deleterious influence upon the growth of bacteria. This can be readily demonstrated by putting a particle of the substance in question in a sterile Petri plate and pouring over it a rather heavily inoculated tube of melted agar (cooled to 45° C. before inoculation). After incubation, any inhibition becomes apparent by a bacterial-free zone surrounding the particle. The thymol-zinc oxid pulp capping shows this phenomenon extremely clearly. In so testing dental cements allowance must be made for the antiseptic action of any uncombined acid. Miller¹ tested in this way copper amalgam, gold amalgam, oxychlorid of zinc, oxyphosphate of zinc, gutta-percha, gold, tin, and tin-gold. Copper amalgam alone showed any constant, significant inhibiting power. Practically no inhibition was exerted by the other substances except gold which gave very inconsistent results. In the case of gold, annealing completely removed this property. There exists a wide-spread belief in the dental profession that tin possesses distinctive antiseptic properties. This is advanced as one of the chief reasons for the use of this material in deciduous teeth. Whatever be the clinical or empiric justification for this procedure, it does not lie in the inhibitory property we are now discussing. Miller, as seen above, found no such property in the case of tin and the author has repeatedly confirmed this observation.

Staub² in similar experiments found mercury most effective in interfering with bacterial growth. In certain tests, silver came second and copper third; in other tests, the positions of silver and copper were reversed. No action was seen with either tin or gold. From these results the idea suggested itself to Staub that finely powdered copper and silver would give good results if incorporated in pastes used for filling the root canals of teeth where discoloration was not contraindicated by cosmetic reasons.

Leist's³ findings differed little from those of Miller and Staub; that is, that copper amalgam has a marked inhibitory action while silver and mercury occupied second place, exhibiting about equal power. Leist's work is also of interest as indicating that the sul-

¹ Dent. Cosmos, 1889, **31**, 917.

² Schweiz. Vrtljhschr. f. Zahnk., 1921, **31**, 36.

³ Ztschr. f. Stomat., 1923, **7**, 414.

phocyanid content of the saliva enhances this inhibitory property of the metals in question. The bacteria-free zone surrounding a silver plate was repeatedly found to be 0.5 to 1.5 mm. greater (wider?) in plates in which the agar contained 0.1 per cent KSCN than in plates in which careful effort had been made to have all the conditions the same except that the agar was devoid of this salt.

Hogeboom and Hurst¹ reported some observations on the bacteriostatic or bactericidal action of certain dental materials. Copper amalgam and copper cement showed large bacteria-free zones. Silver cements and zinc cements were surrounded by small areas. Silicate cement and 22-karat gold plate showed none.

Hattayasy² tested *in vivo*, *i. e.*, in the case of actually filled cavities, the sterilizing effect of filling materials on dentin. Copper amalgam was most effective, and then in order, gold, silver amalgam, tin, gutta-percha and cement.

Pryor³ develops a very interesting phase of this subject. To test the inhibitory action of certain metals, he burnished metal foil to one-half of the palatal surface of a rubber denture and then after this had been worn for a given time cultures were taken from the metal surface and from the rubber surface, for comparison. The foils used were of tin, 24-karat gold, 20-karat gold, aluminum, platinum and silver. Although Hogeboom and Hurst found no inhibitory action manifested by 22-karat gold plate, Pryor observed very marked inhibition with 20-karat gold foil. The cultures from all the metal surfaces with the probable exception of aluminium yielded fewer colonies than the corresponding controls from the rubber surface. Pryor interprets this as involving in part a bacteriostatic or bactericidal action exerted by the metals, which fact is an additional reason in favor of the wider employment of metal bases for dentures.

No unanimity of opinion exists upon the mechanism by which certain metals exert the inhibitory action upon bacterial growth, to which reference has been made in the few preceding paragraphs. In some instances it may be a simple question of the metal going into solution in the medium, in others the possibility has been suggested that the effect is ascribable to atmospheric gases, *e. g.*, O₂, NH₃, CO₂, adsorbed at the metal surface in large quantities and given off into the medium. This bacteriostatic or bactericidal effect of metals, operative apparently at a distance or in very minute quantities, is known as the *oligodynamic* property.

¹ Pacific Dent. Gaz., 1923, **31**, 515; Internat. Jour. Orthodont., 1924, **10**, 214.

² Oestr. ung. V. f. Zahnk., 1898, **14**, 48.

³ Bacterial Growths on Artificial Dentures, Jour. Am. Dent. Assn., 1924, **11**, 195.

INSTRUMENT STERILIZATION.

Solutions of various antiseptics or germicides have been applied to the sterilization of surgical and dental instruments. Substances employed apparently in the gaseous state, as for example, formic aldehyd (formalin or formaldehyd) once so popular in dentistry, belong to this category for they are inactive except in the presence of moisture, *i. e.*, unless the condition is met that they be in solution. Modern surgery as introduced by Lister indeed was an *antiseptic* surgery, dependent largely upon more or less crude phenol.

With the experience that has been gained since the latter years of the seventh decade of the nineteenth century, chemical sterilization has been less and less used until at the present time, except for a few special purposes, instruments are sterilized by heat with or without water as a vehicle for the thermal energy. (For thermal sterilization, see the section on the Influence of Heat on Bacteria.)

The following experiments serve to illustrate why practice has gone in this direction.

- (a) This is a control.
 - 1. With scaler scrape teeth.
 - 2. Wipe off with towel, cotton, or bibulous paper.
 - 3. Inoculate over surface of agar slant.
 - 4. Incubate.
- (b)
 - 1. With scaler scrape teeth.
 - 2. Wipe off with towel, cotton or bibulous paper.
 - 3. Dip in alcohol (95 per cent ethyl).
 - 4. Ignite.
 - 5. When flame is extinguished inoculate over surface of agar slant.
 - 6. Incubate.
- (c)
 - 1. Fill for height of ca. 2 inches, sterile empty tube with 4 per cent phenol.
 - 2 and 3. Same as 1 and 2 in *a* and *b*.
 - 4. Immerse scaler in 4 per cent phenol for one minute
 - 5. Rinse scaler in tube of sterile water.
 - 6. Inoculate surface of agar slant.
 - 7. Incubate.
- (d) Same as *c* except immerse instrument in phenol for two minutes.
- (e)
 - 1. Get water boiling.
 - 2 and 3. Same as 1 and 2 in *a* and *b*.
 - 4. Immerse instrument in boiling water for one minute.

5. Inoculate surface of agar slant.

6. Incubate.

(f) Same as *c* except immerse in boiling water for two minutes.

(g) (*h*) and (*i*). Same as *c* and *d* except in the case of *g* and *h* substitute 1 per cent lysol respectively for one minute and for two minutes; and in the case of *i*, substitute concentrated NaOH for five minutes.

On the day following the results should be noted and correlated.

The following table summarizes the work of student sections in the laboratory. The total number of tests upon which these percentages are based is too low to warrant absolute confidence. The values of the percentages are to be regarded merely as suggestive. The existence and dangers of experimental errors are illustrated by comparing the percentages for *c* (4 per cent phenol for one minute) and *d* (4 per cent phenol for five minutes). According to this, a five-minute exposure fails to do what a one-minute exposure does, which of course is illogical.

	Growth.	No growth.	Per cent with no growth.
(a)	25	1	3.8 +
(b)	4	18	81.0 +
(c)	1	20	95.0 +
(d)	1	17	94.0 +
(e)	2	19	95.0 +
(f)	0	21	100.0
(g)	6	15	71.0 +
(h)	1	22	95.0 +
(i)	1	17	94.0 +

From this table, it is apparent that dental instruments after use need sterilization (Test *a*). This is probably the most important conclusion. It is possible that the bacteria found in the *a* series of tests were not all pathogenic, but some of them certainly were and it is practically easier to sterilize routinely rather than absurdly resort in each case to a determination of the pathogenicity or non-pathogenicity of each bacterial strain recovered. Inspection of the table further shows that immersion in 95 per cent ethyl alcohol and ignition, *b*, and 1 per cent lysol for one minute, *g*, are decidedly unreliable. To illustrate the unreliability of alcohol, reference may be made to the report of Nye and Mallory.¹ The occurrence of two postoperative deaths within forty-eight hours at the Boston City Hospital, from infection with the sporogenic, *B. aërogenes capsulatus*, led to their study. The surgical instruments had been placed in 70 per cent alcohol for five to twenty minutes. It was found that

¹ Boston Med. and Surg. Jour., 1923, 189, 561.

immersion in this solution for one hour failed to destroy bacilli belonging to the *B. aërogenes capsulatus* group. It may be mentioned parenthetically that it is generally accepted that 70 per cent alcohol possesses greater germicidal action than higher or lower concentrations. All of the other methods are on the data afforded, about equally effective and practically satisfactory. The only method which has so far yielded perfect results (no growth in 100 per cent of trials), is boiling water for two minutes.

The use of dichloramin-T as suggested by Prinz¹ for the sterilization of cotton, paper or gutta-percha root-canal points is perfectly reliable. The points are contained in a shallow, wide-mouthed glass bottle, with a top that will screw on tightly. To the inside surface of the top is glued a section of cotton roll. On this from time to time are placed a few drops of a solution of dichloramin-T. Two items need attention: (1) The points must be exposed for a sufficient time, and (2) the disinfectant must be replenished at frequent enough intervals.

These considerations serve to illustrate the need that any and all procedures applied for the sterilization of instruments and materials in the office should be tested out for their effectiveness *under the conditions obtaining in each individual office*. This generalization deserves emphasis, for slight and even unrecognized departures from a described method may render such a modification useless.

¹ Dent. Cosmos, 1918, **60**, 1079.

CHAPTER IV.

CLASSIFICATION OF THE BACTERIA.

BEFORE 1858 biological classification was a convenient yet arbitrary system of indexing. With Darwin's ideas a previously unsuspected meaning came as the dominant motive into classification. The ideal was (and has remained) to represent genetic relationship. To state that this ideal has been realized in bacteriology would be either hyperbole or a confession of ignorance. The unknown is still too vast to permit much more than a classification of convenience. On this account the taxonomy and still more the nomenclature of bacterial species is in a very confused, not to say chaotic, state. This condition has been enhanced by the fact that bacteriology in all its various fields has been developed almost without exception from a strictly utilitarian standpoint. It has been studied in its relations to agriculture, to industry and to disease, as a means to an end and not the end itself, whereas the discovery of a consistent and satisfactory system of classification requires a certain degree of abstraction, such as is obtained by regarding the material in the spirit of pure rather than applied science. Many bacteriologists have been unfamiliar with, and even impatient of, taxonomic aims, methods and conventions. And it is even now uncertain to what extent the concepts of the taxonomist, which have proved useful in other living groups, are applicable to the bacteria. When we speak of a *species* of bacteria we are not sure that we mean the same thing as when we speak of a *species* of algæ or of bryophytes (mosses). Even assuming that these concepts may be projected unchanged upon the bacteria, we find the binomial formality of Linnaeus has been frequently ignored. Due to the impress of Linnaeus, the founder of the current classification of plants and animals, it has been accepted as desirable that each species should be scientifically designated by two, and only two, names; the generic one, which comes first and the specific one which follows. For example, the scientific name of the dog is *Canis familiaris*, *Canis* designates the genus and *familiaris* designates the species.

On account of the various reasons given in the above paragraph, the beginner in bacteriology instead of finding for each bacterial species a single, universally accepted binomial term, usually finds

that he must acquaint himself with several names for each species. These several designations are purely arbitrary and lead to much unfortunate confusion. In the present state of bacteriology—this is unavoidable but it should serve at least one good end by emphasizing the desirability for one universally valid system of classification and nomenclature.

At the present time the classification which is most widely used in this country is that presented in Chester's *Manual of Determinative Bacteriology*, which in turn is largely based on Migula's *Das System der Bakterien*, 1897. We shall present Chester's classification slightly modified and then a more recent and biologically more satisfactory one, contained in Bergey's *Manual of Determinative Bacteriology*.

A. CLASSIFICATION ADAPTED FROM MIGULA AND CHESTER.

ORDER I. EUBACTERIACEÆ: TRUE BACTERIA.

FAMILY 1. *Coccaceæ*.—Cells spherical, division in one, two or three directions, no endospores.

Genus. Micrococcus.—Grouped singly, division in two directions, no flagella, non-motile.

Genus. Sarcina.—Grouped in packets, division in three directions, no flagella, non-motile.

Genus. Streptococcus.—Grouped in chains, division in one direction, no flagella, non-motile.

Genus. Planococcus.—A motile micrococcus.

Genus. Planosarcina.—A motile sarcina.

FAMILY 2. *Bacteriaceæ*.—Short or long rods, motile or non-motile. Endospores present or absent; occur singly in pairs or in chains.

Genus. Bacterium.—Not flagellated, non-motile.

Genus. Pseudomonas.—Flagella polar; monotrichic or lophotrichic.

Genus. Bacillus.—Flagella peritrichic. (around cell).

FAMILY 3. *Spirillaceæ*.—Spirally curved.

Genus. Spirosoma.—Cells curved, rigid, without flagella.

Genus. Microspira.—Cells curved, with one or two polar flagella.

Genus. Spirillum.—Cells curved, with a bundle of polar flagella.

Genus. Spirochæta.—Slender coiled filaments, motile, no flagella.

FAMILY 4. *Mycobacteriaceæ*.—Short or long rods, clavate and cuneate, striated, beaded or showing granules, at times present buds or short branches, some acid-fast (*Mycobacterium*) other not acid fast, (*Corynebacterium*), or as long branched filaments (*Streptothrix*)..

Genus. *Mycobacterium*.

Genus. *Actinomyces* or *Streptothrix*.

FAMILY 5. *Chlamydobacteriaceæ*.—Sheathed bacteria, filamentous.

Genus. *Leptothrix*.—Slender unbranched filaments.

Genus. *Phragmidiothrix*.—Filaments with delicate sheath.

Genus. *Cremothrix*.—Filaments with thicker sheath.

Genus. *Cladothrix* (or *Sphærotilus*).—False branching filaments.

ORDER II. THIOBACTERIACEÆ. HIGHER BACTERIA, SULPHUR AND IRON CONTAINING BACTERIA.

B. CLASSIFICATION ADAPTED FROM BERGEY'S MANUAL, 1925 (BASED ON THE STUDIES OF A COMMITTEE OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS).¹

The following classification omits many taxonomic groups which are of little or no interest to the student of human diseases. The prefixed Roman numerals are retained from, and represent the sequence in, Bergey's *Manual*. This classification, although presenting many novelties to the older student of bacteriology, is regarded by the writer as an important step in the right direction. The inclusion of the spirochetes among the bacteria is tentatively permissible in the present state of our knowledge. The Committee recognized six orders: The Eubacteriales, Actinomycetales, Chlamydomycetales, Thiobacteriales, Myxomycetales and Spirochætales, which all seem to represent natural groups.

CLASS SCHIZOMYCETES.

ORDER I. EUBACTERIALES.

Family I. Nitrobacteriaceæ.

Family II. *Coccaceæ* (Zopf) (Migula).—Cells in their free conditions spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division,

¹ Through the kindness of Dr. D. H. Bergey I have been allowed access to the sheet proofs of the second edition of his *Manual*.

they are frequently flattened in the plane of division, and occur singly, in pairs, tetrads, packets, chains or in irregular masses. Motility rare. Endospores absent. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates. Pigment often produced.

TRIBE I. STREPTOCOCCÆ (Trevisan).—Parasites (thriving only or best on or in the animal body) except genus *Leuconostoc*. Grow well under anaërobic conditions. Many forms grow with difficulty on serum-free media, none very abundantly. Planes of fission usually parallel, producing pairs of short or long chains, never packets. Pigment, if any, white or orange.

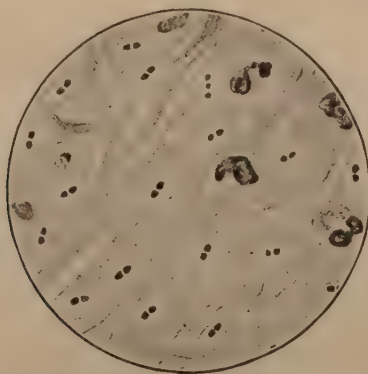


FIG. 10.—*Pneumococcus* in sputum. $\times 1000$. (Kendall.)

Genus I. Diplococcus (Weichselbaum). (Fig. 10).—Parasites, growing poorly or not at all on artificial media. Cells usually in pairs or somewhat elongated cells, encapsulated, sometimes in chains. Gram-positive. Fermentative powers high, most strains forming acid in dextrose, lactose, sucrose and inulin.

The type species is *Diplococcus pneumoniae* (Weichselbaum). An important cause of lobar pneumonia.

Genus II. Streptococcus (Rosenbach) (Fig. 11).—Chiefly parasites. Cells in pairs or in short or long chains, never in packets. Generally Gram-positive. Capsules rarely formed. Do not form zoögleal masses. Grow as effused, translucent, often small isolated colonies on agar streak. In stab cultures little surface growth is developed. Many carbohydrates are fermented with formation of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates. Some species lake blood, others produce

methemoglobin, while a smaller number are without action on blood. (See chapter XVIII for further details).

The type species is *Streptococcus pyogenes* (Rosenbach).

TRIBE II. NEISSERIÆ. Committee S. A. B., 1920.—Strict parasites, some species failing to grow or growing poorly on usual media. Gram stain varies. Growth fairly abundant on serum media. Cells normally in pairs.

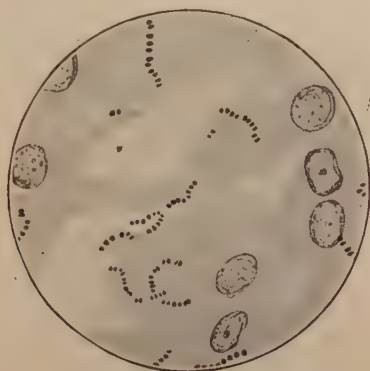


FIG. 11.—Streptococci in liver, section stained by Gram's method. $\times 800$. (Kolle and Hetsch.)

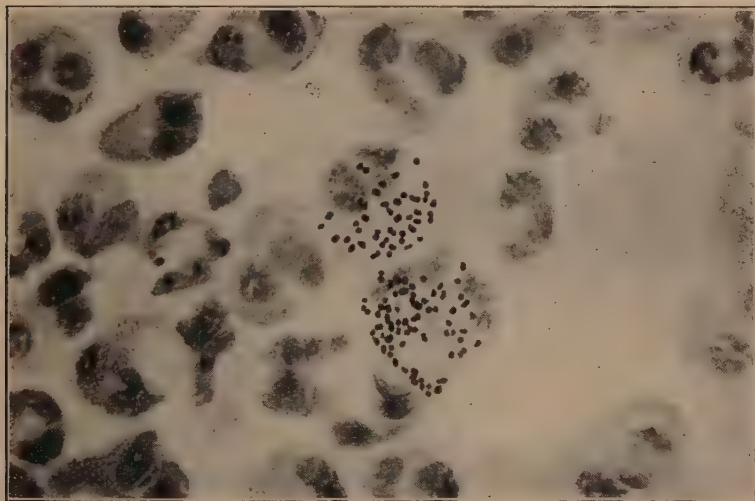


FIG. 12.—*Neisseria gonorrhoea*. (Kendall.)

Genus IV. Neisseria (Trevisan).—Characters, those of the tribe. *N. gonorrhoea*, cause of gonorrhea. *N. catarrhalis*, weakly pathogenic, frequently in the mouth. (Figs. 12 and 13).

Genus V. Gaffkya (Trevisan).—Parasitic, occurring in the animal body and in special media as tetrads, while in ordinary

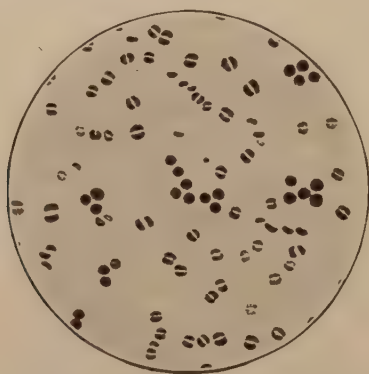


FIG. 13.—*Neisseria catarrhalis* and staphylococcus. (Kendall.)

culture media they occur in pairs and irregular masses. Gram-positive. The type species is *Gaffkya tetragena*, known commonly as *Micrococcus tetragenus*. (Fig. 14.)

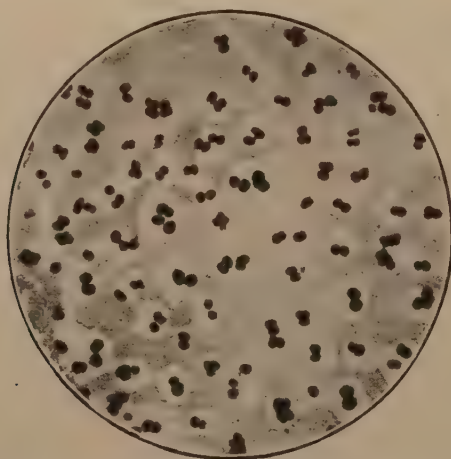


FIG. 14.—*Gaffkya tetragena* from peritoneal fluid. Stained with fuchsin. $\times 1000$ diameters. (Fränkel.)

TRIBE III. MICROCOCCÆ (Trevisan).—Facultative parasites or saprophytes. Thrive best under aërobic conditions. Grow well on artificial media, producing abundant surface growth. Planes of fission often at right angles. Occurring singly and in pairs and

in cell aggregates, groups or packets. Generally stained by Gram. Many species form yellow or red pigment.

Genus VI. Staphylococcus (Rosenbach) (Fig. 15).—Usually parasitic, cells occur singly and in pairs and in irregular groups, rarely in packets. Usually Gram-positive. Growth fair to good on the surface of artificial media. As a rule carbohydrates are fermented with the formation of acid. Gelatin commonly liquefied. Nitrates may or may not be reduced. (Produce hemolysis on blood agar.) Pigment white or orange, or less commonly lemon-yellow.

The type species is *Staphylococcus aureus* (Rosenbach).

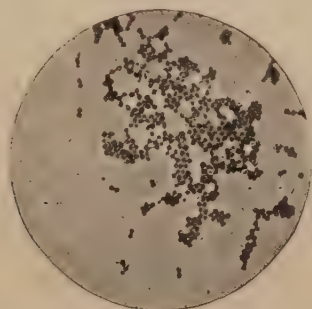


FIG. 15.—*Staphylococcus*. $\times 1100$. (Park and Williams.)

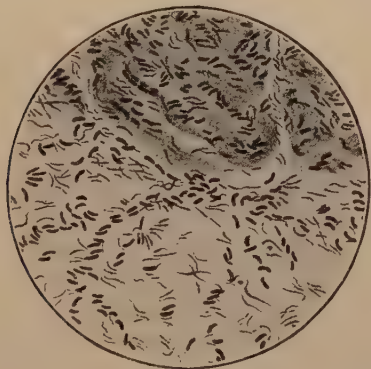


FIG. 16.—Cholera vibrios from feces. (Kendall.)

Family III. Spirillaceæ (Migula).—Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous, usually without endospores. As a rule, motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

Genus I. Vibrio (Mueller).—Cells short, bent rods, rigid, single or united into spirals. Motile by means of a single (or rarely, two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aërobic, facultative anaërobic. No endospores formed. Usually Gram-negative. Water forms; a few parasites.

The type species is *Vibrio comma* (Schroeter). (Fig. 16.)

Family IV. Bacteriaceæ (Cohn).—Rod-shaped cells without endospores. Motile or non-motile. Metabolism complex, amino-acids being utilized, and generally carbohydrates. Usually Gram-negative.

TRIBE I. CHROMOBACTEREÆ. Committee S. A. B., 1920.—Water and soil bacteria producing a red, yellow, violet or blue-green pigment.

Genus II. Pseudomonas (Migula).—Principally water and soil bacteria producing a water-soluble pigment which diffuses through the medium as green, blue or yellowish-green. Motile or non-motile. Gram-negative.

The type species is *Pseudomonas aeruginosa* (Schroeter) (Migula).

TRIBE IV. LACTOBACILLEÆ. Committee S. A. B., 1920.—Rods, often long and slender. Gram-positive. Non-motile. Without endospores. Generally produce acid, as a rule lactic, from carbohydrates. When gas is formed it is CO₂ without H₂. Usually somewhat thermophilic. As a rule microaërophilic. Surface growth on media is poor.

Genus VII. Lactobacillus (Beijerinck).—Generic characters are those of the tribe.

L. bulgaricus: No gas in carbohydrate media, maltose not fermented, mannitol acid.

L. acidophilus: No gas in carbohydrate media, acid in maltose, acid in lactose, mannitol not fermented, acid in raffinose, dextrin not fermented.

TRIBE VII. BACTERIÆ. Committee S. A. B., 1920.—Gram-negative rods occurring commonly in the intestinal tract of animals. Grow well on artificial media. Many species attack carbohydrates, forming acid or acid and gas. Relatively few species liquefy gelatin. When motile, the flagella are peritrichous.

Genus XI. Escherichia (Castellani and Chalmers) (Fig. 17).—Motile or non-motile rods, commonly occurring in the intestinal canal of normal animals. Attack numerous carbohydrates forming acid and frequently acid and gas. Do not produce acetyl-methyl-carbinol,

The type species is *Escherichia coli* (Escherich) (Castellani and Chalmers).

Genus XIV. Salmonella (Lignières).—Motile forms. Generally occur in the intestinal canal of animals in various types of acute inflammatory conditions. Attack numerous carbohydrates with the formation of both acid and gas. In general do not form acetyl-methyl-carbinol.

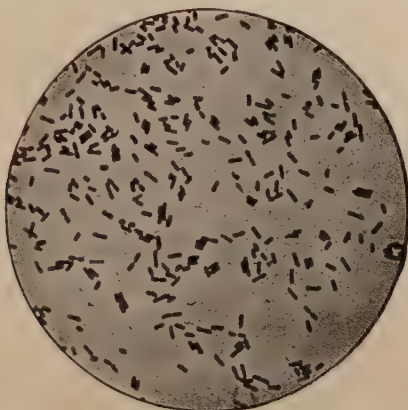


FIG. 17.—Colon bacilli. Twenty-four-hour agar culture. $\times 1100$. (Park and Williams.)

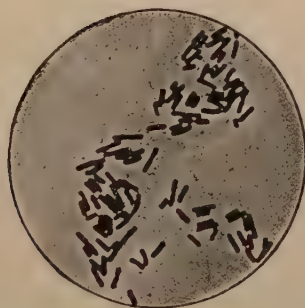


FIG. 18.—Typhoid bacilli from nutrient gelatin. $\times 1100$. (Park and Williams.)

The pathogenic species include: *S. enteritidis*, *S. schottmülleri*, *S. paratyphi*, and *S. aërytrycke*.

Genus XV. Eberthella (Castellani and Chalmers) (Fig. 18).—Motile or non-motile rods, generally occurring in the intestinal canal of man, usually in different forms of enteric inflammation. Attack a number of carbohydrates with the formation of acid but no gas. Do not form acetyl-methyl-carbinol.

The type species is *Eberthella typhi* (Eberth-Gaffky) (Castellani and Chalmers). Cause of typhoid or enteric fever.

TRIBE VII. PASTEURELLÆ (Castellani and Chalmers).—Gram-negative rods showing bipolar staining. Parasitic forms with slight fermentative powers.

Genus XVII. Pasteurella (Trevisan).—Aërobic, facultative. No gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in other animals.

P. pestis, the cause of bubonic and pneumonic plague in man. Fig. 19.

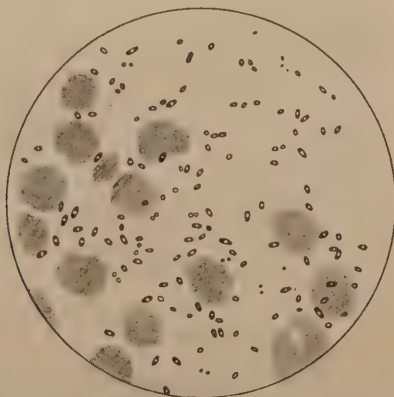


FIG. 19.—*P. pestis* in smear from acutely inflamed gland. (Park and Williams.)

TRIBE IX. KLEBSIELLEÆ (Trevisan).—Short rods, somewhat plump with rounded ends, mostly occurring singly. Encapsulated. Non-motile. Gram-negative. Ferment a number of carbohydrates with formation of acid and gas. Encountered principally in respiratory tract of man. Aërobic, growing well on ordinary culture media. Gelatin not liquefied.

Genus XVII. Klebsiella (Fig. 20).—Generic characters those of the tribe. The type species is *K. pneumoniae* (Friedländer's bacillus).

TRIBE X. HEMOPHILEÆ, Committee S. A. B., 1920.—Minute parasitic forms growing only in the presence of hemoglobin, ascitic fluid or other body fluids, or in the presence of certain growth-accessory substances found in sterile, unheated plant tissue (potato). Non-motile. Gram-negative.

Genus XIX. Hemophilus, Committee S. A. B., 1917.—Minute rod-shaped cells, sometimes thread-forming and pleomorphic.

Non-motile. Strict parasites growing best (or only) in the presence of hemoglobin and in general requiring blood serum, ascitic fluid, or certain growth accessory substances. Gram-negative.

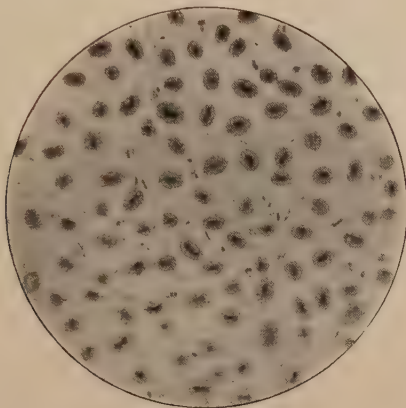


FIG. 20.—*Klebsiella pneumoniae* stained for capsule by Huntoon's method. $\times$ about 900. (Huntoon.)

The type species is *Hemophilus influenzae* (Pfeiffer) Committee S. A. B. Regarded at present as probably a secondary invader and not the primary organism in influenza.

Genus XX. Dialister.—Minute rod-shaped cells, occurring singly, in pairs and short chains. Non-motile. Strict parasites. Growth occurs only under anaërobic conditions in media containing fresh, sterile tissue or ascitic fluid.

The only species known is *Dialister pneumosintes* (Olitsky and Gates). Possibly the primary organism in influenza.

TRIBE XI. BACTEROIDEÆ (Castellani and Chalmers).—Motile or non-motile rods, without endospores. Show good growth on ordinary media, without pigment formation. Obligate anaërobes.

Genus XXI. Bacteroides.—The characters of the genus are those of the tribe. The species include *B. fragilis* (Veillon and Zuber) and *B. bifidus*.

Family V. Bacillaceæ (Fischer).—Rods producing endospores, usually Gram-positive. Flagella, when present, peritrichous. Often decompose protein media actively through the agency of enzymes.

Genus I. Bacillus (Cohn) (Figs. 21, 22 and 23).—Aërobic forms. Mostly saprophytes. Generally liquefy gelatin. Often occur in long chains and form rhizoid colonies. Form of rod usually not greatly changed at sporulation.

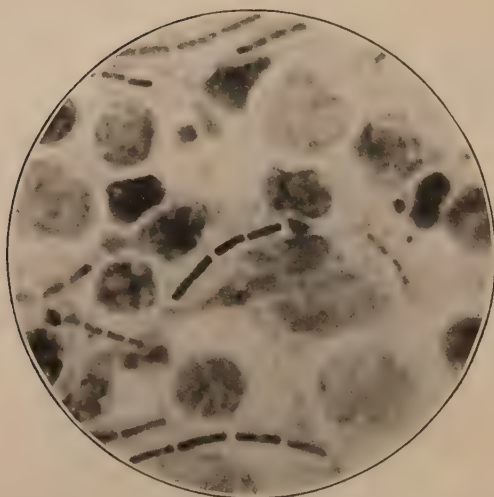


FIG. 21.—Spread from the liver of a mouse dead of anthrax septicemia. $\times 1700$. Stained by Williams' rabies stain. Shows capsule. (Gerber and Siegel.)

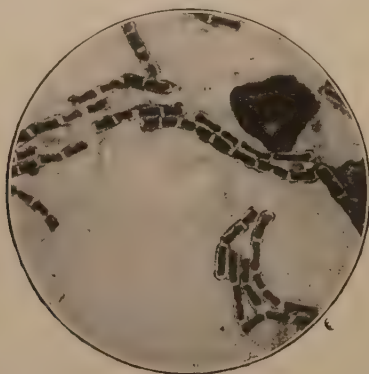


FIG. 22.—*Bacillus anthracis*, showing capsule formation. $\times 1000$. (Kolle and Hetsch.)

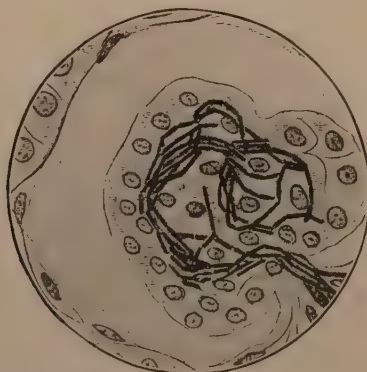


FIG. 23.—*Bacillus anthracis*, section from kidney, semidiagrammatic. $\times 500$. (Kolle and Hetsch.)

The type species is *Bacillus subtilis* (Cohn). *B. anthracis* (cause of anthrax).

Genus II. Clostridium (Prazmowski) (Fig. 24).—Anaërobes or microaërophiles, often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* (Prazmowski). *C. tetani* (cause of tetanus) also *C. welchii* and the bacteria of gas-gangrene.



FIG. 24.—Tetanus bacilli with spores distending ends. $\times 1100$. (Park and Williams.)

ORDER II. ACTINOMYCETALES (BUCHANAN).

Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. No pseudoplasmodium. No deposits of free sulphur or iron. No bacteriopurpurin. Endospores not produced but conidia are developed in some genera. Usually Gram-positive. Non-motile. Some species parasitic in animals or plants. As a rule strongly aërobic (except for some species of *Actinomyces* and the genera *Fusiformis* and *Leptotrichia*) and oxidative. Complex proteins frequently required. Growth on culture media often slow; some genera showing mould-like colonies. No water forms.

Family I. Actinomycetaceæ (Buchanan).—Filamentous forms often branched and sometimes forming mycelia. Conidia sometimes present. Some species are parasitic.

Genus II. Leptotrichia (Trevisan).—Thick, long, straight or curved filaments, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Filaments fragment into short, thick rods. Anaërobic or facultative. No aërial hyphæ or conidia. Parasites or facultative parasites.

The type species is *Leptotrichia buccalis* (Robin) (Trevisan).

Genus III. Actinomyces (Harz) (Figs. 25 and 26).—Organisms growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Sometimes parasitic, with clubbed ends of radiating threads conspicuous in lesions in the animal body. Some species are microaërophilic or anaërobic. Non-motile.

The type species is *Actinomyces bovis* (Harz).

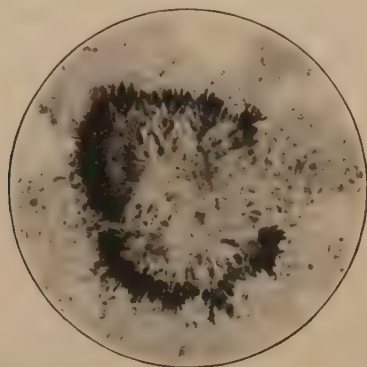


FIG. 25.—*Actinomyces* colony, showing peripherally arranged clubs. (Kendall.)

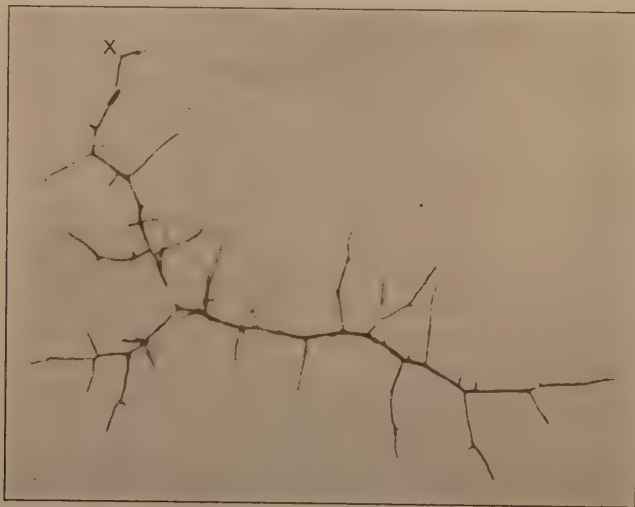
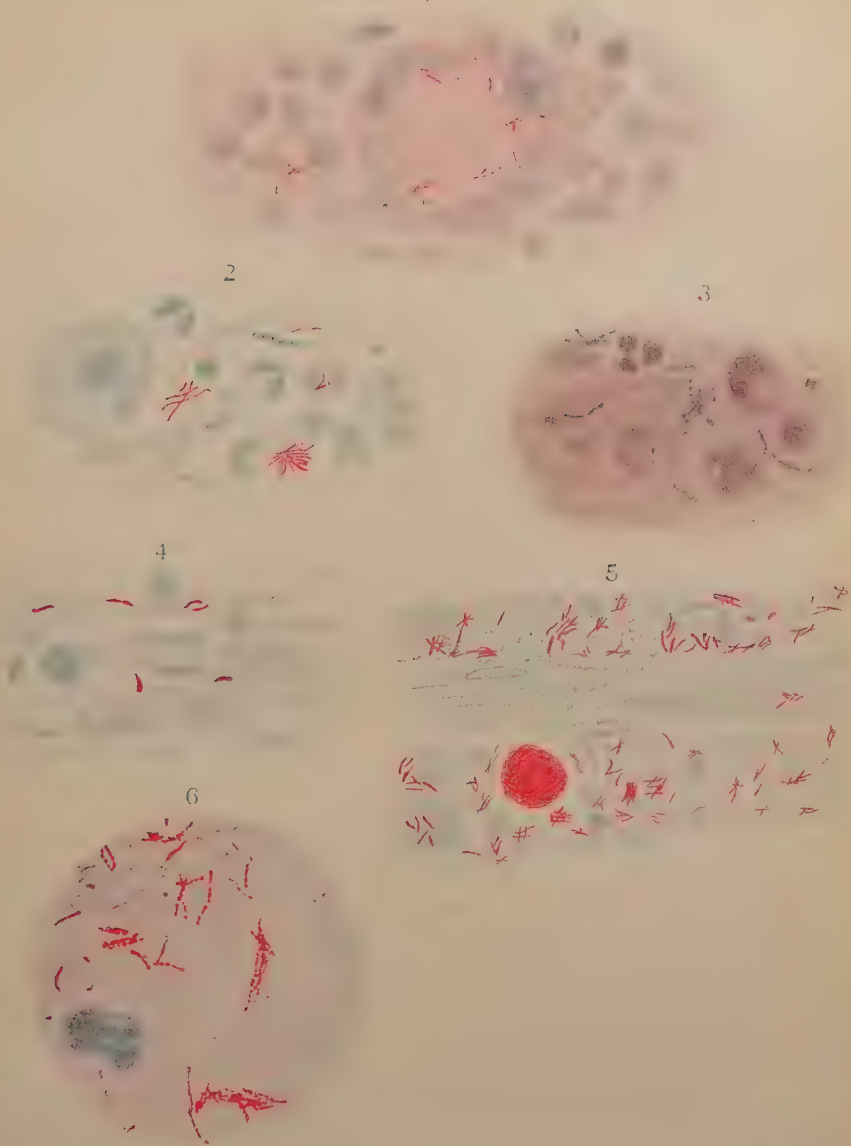


FIG. 26.—Branching filament from potato culture. $\times 1000$. (Steele.)

Family II. Mycobacteriaceæ (Chester).—Parasitic forms. Rod-shaped, frequently irregular in form but rarely filamentous and with only slight and occasional branching. Often stain unevenly (show-

PLATE I



Courtesy of Park and Williams

FIG. 1.—Tuberculous lymph node "giant cell" containing tubercle bacilli "human type." Bacilli red, rest of specimen blue. Ziehl-Neelsen stain. $\times 1000$ diam.

FIG. 2.—Tuberculous sputum from human case. Stain same as above. $\times 1000$ diam.

FIG. 3.—Tuberculous sputum, human case. Stained by Hermann's method. Tubercle bacilli violet, rest of specimen brown.

FIG. 4.—Pus from tuberculous abscess in cow. "Bovine type" of bacillus. Stained same as Figs. 1 and 2. $\times 1000$ diam.

FIG. 5.—Section through leprous skin showing bacilli in clumps in and out of cells and large "leprous cell" containing a ball of bacilli. Stained with Ziehl-Neelsen.

FIG. 6.—Photograph of human type of tubercle bacilli from sputum. Bacilli in red, rest of specimen blue. $\times 1000$ diam. (Fränkel and Pfeiffer.)

ing variations in staining reaction within the cell). No conidia formed.

Genus I. Mycobacterium (Lehmann and Neumann) (Plate I).—Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched forms. Growth on media slow. Aërobic.

Several species pathogenic to animals.

The type species is *Mycobacterium tuberculosis* (Koch) (Lehmann and Neumann).

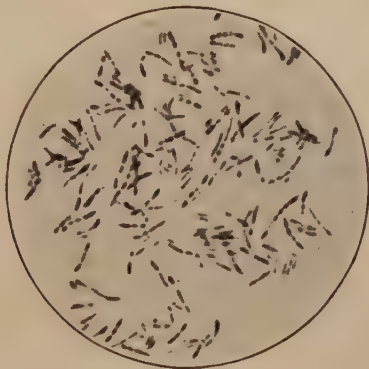


FIG. 27.—*Corynebacterium diphtheriæ*, methylene-blue stain. $\times 1000$. (Kendall.)

Genus II. Corynebacterium (Lehmann and Neumann) (Fig. 27).—Slender, often slightly curved, rods with a tendency to club and pointed forms, with branching forms in old cultures. Barred, uneven staining. Not acid-fast. Gram-positive. Non-motile. Aërobic. No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

The type species is *Corynebacterium diphtheriæ* (Loeffler) (Lehmann and Neumann).

Genus III. Fusiformis (Hoelling) (Fig. 28).—Obligate parasites. Anaërobic or microaërophilic. Cells frequently elongate and fusiform, staining somewhat unevenly. Filaments sometimes formed; non-branching. Non-motile. No spores formed. Growth in laboratory media feeble.

F. dentium is the fusiform bacillus of Vincent's infection.

Genus IV. Pfeifferella (Buchanan).—Non-motile rods, slender, Gram-negative, staining poorly, sometimes forming threads and showing a tendency toward branching. Gelatin may be slowly

liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

The type species is *Pfeifferella mallei* (Loeffler) (Buchanan), the cause of glanders.

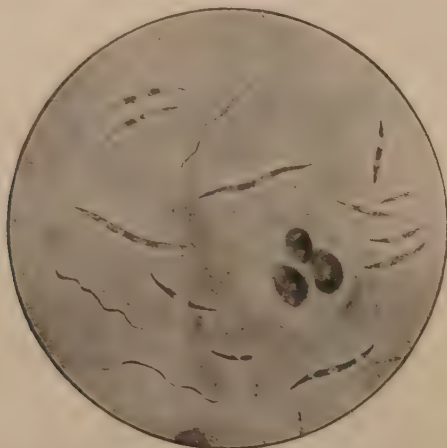


FIG. 28.—Vincent's fusiform bacillus with accompanying spirochetes. (Park and Williams.)

ORDER III. CHLAMYDOBACTERIALES (BUCHANAN).

Filamentous bacteria, alga-like, typically water forms, frequently sheathed, without true branching although false branching may be present. The sheath is frequently impregnated with iron. Conidia may be developed, but never endospores. Sulphur granules or bacteriopurpurin never present. Mature cells or filaments not motile nor protozoön-like.

ORDER IV. THIOBACTERIALES (BUCHANAN).

Cells various, typically containing either granules of free sulphur, or bacteriopurpurin, or both, usually growing best in the presence of hydrogen sulphid. The cells are plant-like, not protozoön-like, not producing a pseudoplasmodium or a highly developed resting stage. Spores are rarely or never formed.

ORDER V. MYXOBACTERIALES (BUCHANAN).

Motile, rod-like organisms multiplying by fission, secreting a gelatinous base and forming a pseudoplasmodium-like aggregation

before passing into a more or less highly developed cyst-producing, resting stage in which the rods may become encysted in groups without modification, or may be converted into spore masses.

ORDER VI. SPIROCHAETALES (BUCHANAN).

Protozoön-like in many characters. Cells usually relatively slender, flexous spirals: multiplication of cells apparently by longitudinal division in some types, by transverse division in others, or both.

One family is recognized, Spirochætaceæ.

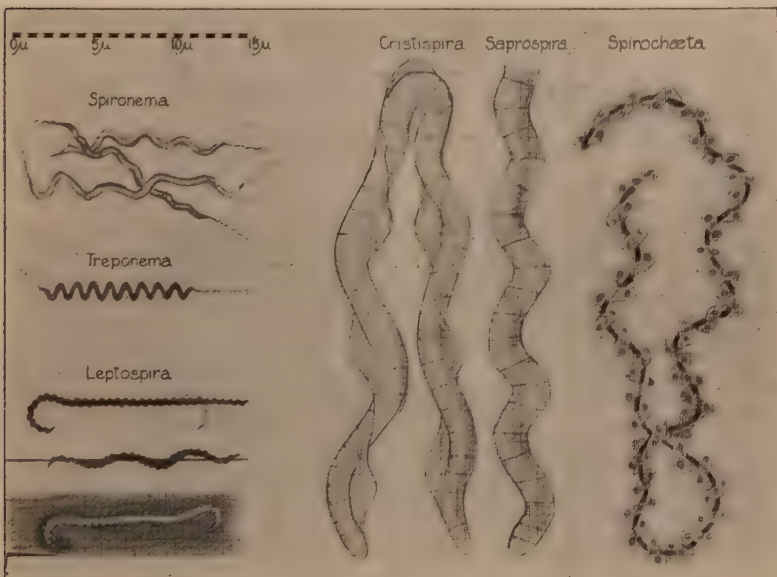


FIG. 29.—Diagram contrasting the characteristic features and relative proportions of spirionema, treponema, cristispira, saprospira, spirocheta and leptospira. The scale in microns is given in the upper left-hand corner of the figure. (Noguchi.)

Family I. Spirochætaceæ (Schwellengrebel) (Fig. 29).—Characters those of the Order.

Genus IV. Borrelia (Fig. 28).—A spiral, flexible body with terminal filaments but no membrane. Spirionema in Fig. 29.

B. recurrentis (Obermeier) (Lebert) and *B. vincenti* (the spirochete of Vincent's infections) belong here.

Genus V. Treponema (Schaudinn) (Figs. 30, 31 and 32).—Parasitic and frequently pathogenic forms with undulating or rigid spirilliform body. Without crista or columella. With or without flagelliform tapering ends,

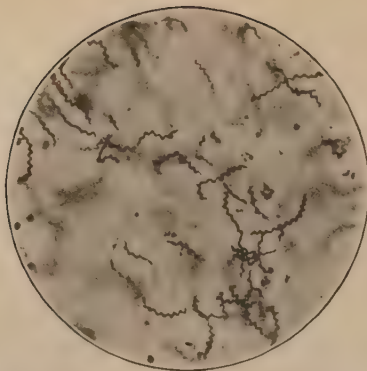


FIG. 30.—*Treponema pallidum*, congenital syphilitic liver. (Kendall.)

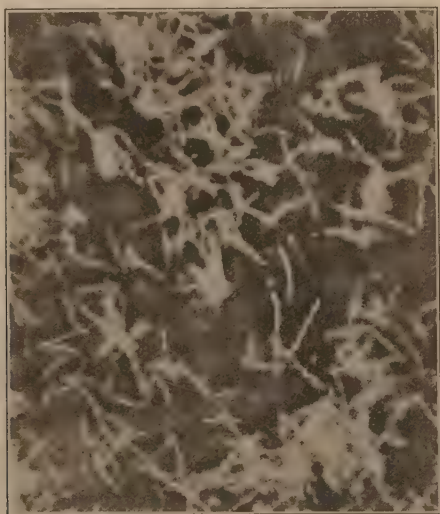


FIG. 31.—*Treponema microdentium* from pure 10-day culture. (Noguchi.)

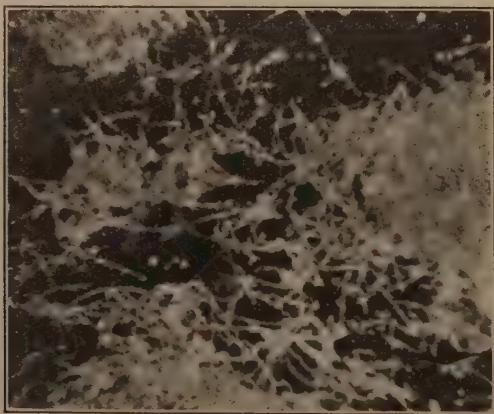


FIG. 32.—*Treponema mucosum* from pure 10-day culture. (Noguchi.)

The type species is *Treponema pallidum* (Schaudinn and Hoffman). *T. microdentium*, *T. macrodentium*, and *T. mucosum*, all of the human mouth, also belong here.

Genus VI. Leptospira (Noguchi). Parasitic forms. Sharply twisted cylinders with flagelliform tapering ends, one extremity being sharply curved into a "hook."

The type species is *Leptospira icthæmorrhagiæ* (Inada and Ido) (Noguchi), the cause of infectious jaundice, *L. icteroides*, probably the cause of yellow fever belongs here.

CHAPTER V.

FILTERABLE VIRUSES.

IN the case of certain infections, if a suspension of infectious material be passed through an unglazed porcelain filter whose pores are so fine that the passage of known bacteria is prevented, the bacteria-free filtrate will be found to have retained its infectivity. There is good reason to believe that this infectivity is due to certain, very minute particulate living beings and to these the term filterable viruses is applied. They are also often described as ultra-microscopical because many of them are undemonstrable by the ordinary microscope, but this term is not strictly correct.

Nothing is really known regarding the biological nature of these viruses; whether they be bacteria, protozoa or Haeckel's protista. It is very unlikely that they constitute a homogenous group. Only as a matter of convenience do we in our ignorance classify them together on the basis of their extremely small size. In the case of two filterable viruses, estimates of their size are smaller than 23 to 25 $\mu\mu$ for the virus of chicken plague and about 33 to 36 $\mu\mu$ for the virus of the mosaic disease of tobacco. ($1 \mu\mu = 0.000001 \text{ mm.}$)

There exists naturally a tendency in the case of diseases which are almost certainly infectious and in which efforts to find a bacterial or protozoan agent have so far failed, to look for a filterable virus. The infections of man or other animals, in which the filterable nature of the exciting cause has been established with more or less certainty, include: Rabies, molluscum contagiosum, dengue, warts, pappataci fever, variola, trachoma, epidemic parotitis (mumps), poliomyelitis, measles, alastrim and inclusion blenorrea, at least some types of herpes, encephalitis lethargica, hoof and mouth disease, pleuropneumonia of cattle, chicken plague, epithelioma contagiosum, and hog cholera. The bacteriophage of d'Herelle, if this author's interpretation be accepted, would be classed as a filterable virus pathogenic for bacteria.

It is desirable to point out in this list certain infections which are of professional interest to the dentist, *viz*: Epidemic parotitis, the relation of herpes (*e. g.*, herpes labialis) to encephalitis, and hoof and mouth disease. The virus of herpetic vesicles or a closely

related virus may occasionally be encountered in the saliva of normal individuals.

In considering the filterable viruses, it should not be passed over that certain well-known protozoa or bacteria may at times assume a filterable form, *e. g.*, *Trypanosoma lewisi* (Novy and MacNeal), *Bacterium pneumosintes* of Olitsky and Gates (considered by these authors as of importance in influenza) and its close relatives, and the tubercle bacillus.¹

A very concise and satisfactory summary of the present status of our knowledge on the filterable viruses is given by Simon.²

¹ Valtis: Ann. d l'Inst. Pasteur, 1924, **38**, 458.

² Physiol. Rev., October, 1923, p. 483.

PART II.

INFECTION.

CHAPTER VI.

NATURE OF INFECTION AND GENERAL CONCEPT.

It has long been recognized that "plants and animals remote in the scale of Nature, are bound together by a web of complex relations." A classical, though hypothetical, instance of this "web" is given in Darwin's *Origin of Species*. Abbreviated, it follows. Bumble-bees are necessary for the fertilization of the red clover. If the whole genus of bumble-bees became extinct or very rare in England, the red clover would become very rare, or wholly disappear. The number of bumble-bees in any district depends in a great measure upon the number of field-mice, which destroy their combs and nests. Now the number of mice is largely dependent on the number of cats. "Hence it is quite credible that the presence of a feline animal in large numbers in a district might determine, through the intervention first of mice and then of bees, the frequency of certain flowers in that district."

Adaptation to such, and often more intimate relationships, is required of every living species for continued existence. The ecological conditions are no less exacting and crucial than any other phase of the environment. Great disparity in size between the species involved does not lessen the necessity for, or the difficulty of, adaptation. These organic associations appear under various forms, among which some have been summarized by Hegner¹ when one member of the association is a protozoön. The same categories would hold good for the bacteria.

1. The two members of the association may be mutually and equally beneficial.

2. One member may secure a greater advantage than the other without either undergoing any disadvantage.

¹ Scientific Month., 1924, 19, 140.

3. One member may live more or less at the expense of the other without causing any injury to the body.

4. One member may injure the body of the other but not enough to produce clinical symptoms unless present in large numbers.

5. One member may be pathogenic to the other, *i. e.*, may give rise to a diseased condition. The disease produced may be mild and only a contributing cause of death or may be severe and the direct cause of death. Often one species of parasite may be lethal under certain circumstances and non-lethal under others.

The pathologist is interested chiefly in this last group of associations and also to some extent in the fourth group. The difference is obviously only a quantitative one. These two groups, the fourth and the fifth, constitute our infections. When a microorganism is said to be pathogenic, we mean that we are dealing with an imperfect adaptation. The concept of an infectious disease carries two ideas: (1) This imperfect adaptation, a disturbed equilibrium, and (2) an attempt at adaptation, to regain a state of equilibrium. According to the success with which this latter idea is realized, we have recovery and cure. The symptoms, the anatomical and functional changes, which occur in and characterize an infection, are only the manifestations of this disturbed equilibrium and of the efforts to reestablish an equilibrium.

The demonic possession theory of disease has something which appeals to the frailer side of human Nature. We like to personify what inconveniences or damages us and attribute thereto malicious intent. When bacteria, discrete living beings, were substituted for demons, the difference was more a matter of names to the lay mind and we still talk ordinarily of bacteria as sentient and possessed of diabolical proclivities. Of course, we know better and do not mean just what we say; the anthropomorphism of our expression was to be understood only as a metaphor. But this suppressed reservation is not always conducive to clear thinking; if we do not always say what we think, we sometimes think what we say. It is consequently of the greatest importance in beginning the study of infection to emphasize explicitly that the discomfort or death of the host, attendant upon bacterial infection, is not the conscious objective of the parasite.

In fact, the interests of the parasite would usually best be served by the continued health of the host. The attendant damage to, or death of, the host is an unforeseen and unwelcome event for the parasite. A source of food is destroyed and transference to another host, in which act the bacteria are absolutely passive, is always

hazardous for the continuance of the parasitic species. The ideal condition of parasitism, where adaptation was perfect, would be where the host suffered no harm or at worst no significant handicap. Such a condition we may see actually realized in the case of some typhoid carriers, in whom either with or without a history of clinical typhoid, *Bacillus typhosus* flourishes for years (forty or fifty, it may be) without arousing the suspicions of the host. The condition of the permanent carrier may from the biological or evolutionary standpoint be regarded as the realization of the goal of parasitism, practically perfect adaptation of host and parasite.

When we speak of the adaptation of two species to each other, we must recognize that this process is a mutual one, in which both species are concerned. The host must have developed protection, or an adequate reserve of protective factors, against the incursions of the parasite; and the parasite must have possessed or acquired various properties which permit it to function normally under the conditions offered by the host. Some of the most elementary qualifications of a successful parasite must be: (1) Ability to satisfy its nutritive requirements in the host; (2) to have an optimum temperature range corresponding with the temperature of the host; (3) to have an optimum hydrogen-ion concentration range corresponding with that of the host; (4) to protect itself against distinctly harmful substances and cellular activities of the host. On the other hand, the host must solve the problem of parasitism or succumb. The solution may lie in the establishment of a *modus vivendi* with the parasite as illustrated at times in the permanent carrier condition, a condition which some of our chronic infections may approximate, or in the mobilization of offensive and reparative forces which eliminate or destroy the parasite.

It should be apparent from the above considerations that infection is inherently a two-sided process. We must study the parasite and the host in their mutual relations. Biologically considered, our problem falls in the category of imperfect adaptations; and it is from this view-point that the following pages have been written.

CHAPTER VII.

DISSEMINATION AND TRANSMISSION OF INFECTIOUS AGENTS AND CARRIERS.

DISSEMINATION AND TRANSMISSION OF INFECTIOUS AGENTS.

THE infectious diseases of bacterial, fungous, or protozoan origin, which affect man, are either peculiarly human or have a slightly wider distribution limited to the domestic mammals with which man is more or less intimately associated. The causative agents cannot under natural conditions long survive or materially multiply outside of the bodies of these animals. Few, and if the total damage done be considered, relatively unimportant, exceptions to these statements can be made. Taking them as facts, their bearing upon the transmission of human infections is at once obvious. In seeking for the source of infection in a particular case, we are materially aided by this limitation of possibilities. The source is usually, sometimes always, directly or indirectly another human being.

Another consideration of prime importance for the understanding of the transmission of infectious agents is that the microörganism is a purely passive object in its transference from one individual host to another. This event is characterized by the fortuitousness exhibited by the Biblical seed falling on good or on stony soil.

Inasmuch as most pathogenic microbes find conditions outside of the body of their host unfavorable for their continued existence, and inasmuch as their falling on the good soil of a susceptible individual is purely a matter of chance, the period of transference is one of peculiar hazard for the parasite. Many of the most successful of hygienic measures are effective by simply increasing the hazard to which the microörganism is naturally exposed at this period.

Trauma in the form of scratches, abrasions, puncture, section or laceration, is an ordinary way by which pyogenic infections are transmitted. Anthrax, actinomycosis, tetanus, gas gangrene and related infections usually gain access to the body in this same way. Mechanical injury to the gingivæ during or following dental operations, as from poorly finished margins of fillings or poorly adapted bands or indiscrete methods of separation, opens the way for infec-

tion. Exposure and death of the dental pulp with consequent invasion of the periapical tissues by bacteria may follow attrition of the dental tissues or fracture of the crown. Fatal staphylococcal and streptococcal infections have been implanted at the extraction of the tooth, and this minor operation at times has been followed by the development of lesions of tuberculosis, syphilis and actinomycosis at the site of extraction. The sterilization of all surgical, including dental, instruments is a routine procedure devised solely to avoid traumatic infection. The operator himself, general surgeon or dentist, at times becomes infected from wounds inflicted accidentally while working on an infected patient. Syphilis has been contracted in this way and also more or less severe or even fatal pyogenic infections.

The infections of the respiratory tract, pneumococcal and streptococcal pneumonias, influenza, pneumonic plague, diphtheria, scarlet fever, measles, pulmonary tuberculosis, whooping cough, the various types of coryza, some of the infections of the nasal accessory sinuses, otitis media and mastoiditis, may be acquired by the inhalation of infected dust or droplets, and by the use of contaminated dishes, glasses, towels and handkerchiefs. In this same category probably belong meningococcus meningitis and anterior poliomyelitis. The microbes in the dust are derived from those in sputum, nasal or aural discharges, which have become dried and pulverized. The water droplets are expelled by the sneezing and coughing characteristic of these diseases. The droplets are of all sizes, some microscopical. At the present time the tendency is to minimize the importance of droplet infection and several different diseases usually contracted by inhalation can be safely treated in the same ward if certain simple and common-sense precautions are observed. It is obvious that the dentist by his occupation is likely either to receive or transmit those infections in which the microorganisms are eliminated by the mouth or nose. In passing, it probably deserves noting that some students, prominently Calmette, regard the primary portal for pulmonary tuberculosis to be usually the alimentary instead of the respiratory tract.

Foods, including milk and water, play an important part in the transference of alimentary infections. Water is the chief vehicle for the spread of typhoid fever, Asiatic cholera, and bacillary and amœbic dysentery. The water becomes infective usually from the stools and sometimes from the urine of the patient or carrier. Milk can act in the same way, but although the bacteria can rapidly multiply in this vehicle it is of less importance except in the case

of the summer diarrheas of infancy. Epidemics of streptococcal tonsillitis and diphtheria can also be spread by milk. Ordinary cooking often does not serve to sterilize foods. Cases of "meat-poisoning," caused by paratyphoid and enteritis bacilli, and of botulism following the consumption of sausages, ripe olives, etc., occur in this way. Uncooked foods, as fruits, some vegetables and salads, are likewise capable of spreading gastro-intestinal diseases especially when fertilization with "night-soil," *i. e.*, human excreta, is permitted. The food may come from a diseased animal or be infected at its source or in its distribution or by the cook in its preparation for the table or even by the ultimate consumer himself from contaminated fingers.

Some infections are carried from man to man by the intervention of an arthropod. The ordinary domestic fly (*Musca domestica*) can in a purely mechanical fashion transfer typhoid and allied fevers. Flies are equally attracted to feces, to the kitchen and to the dining table. Infectious material adheres to their feet and legs, and is taken into their alimentary tract whence it may be regurgitated or passed as feces. In the severe typhoid epidemics of the camps in the Spanish-American war, the fly is credited with having played a highly important part. The indispensable role of the mosquito in the transmission of filariasis, malaria, yellow fever and dengue fever is well-known. Other suctorial diptera, *e. g.*, *Glossina palpalis*, and *G. morsitans*, are likewise necessary for the transference of the trypanosomiasis (African sleeping sickness). The blood-sucking habits of certain flies favors the dissemination of these diseases. The patient's blood infects the suctorial apparatus which in turn infects the human beings subsequently "bitten" or the infectious agent, a protozoön, may have to pass through certain stages of its life cycle in the tissues of the fly before this insect becomes capable of transmitting the disease to the second human being. Typhus is unable to make headway without the aid of the louse and bubonic plague is spread by the rat-flea. The flea in whose feces are numbers of *B. pestis* gathered from a meal on a patient or infected rat, bites the prospective patient. The act of biting is usually accompanied by the act of defecation. The prospective patient in his instinctive scratching the site of the bite, rubs the bacteria into his skin and thereby becomes infected.

The venereal diseases, syphilis, gonorrhea and soft chancre as the term indicates, are usually contracted during coitus. About 10 per cent of the cases of syphilis show an extragenital primary localization.

CARRIERS.

The "carrier" in the terminology of infectious diseases is one who in the absence of characteristic or significant clinical symptoms discharges pathogenic organisms into his environment. Such an individual, especially when the condition is unrecognized as often happens, is one of the most important agents in the dissemination of infectious diseases. On that account considerable interest has been attracted to the subject and our present knowledge is adequately set forth in a number of recent publications: (1) Ledingham and Arkwright. *The Carrier Problem in Infectious Disease*, 1912; (2) Simon, *Human Infection Carriers*, 1919; (3) Nichols, *Carriers in Infectious Diseases*, 1922. The significance of the carrier was first appreciated and worked out in the case of typhoid fever. Since then the carrier problem has been found to exist and to constitute a not-to-be-neglected factor in the spread of such diseases as the paratyphoid fevers, bacillary and amoebic dysentery, Asiatic cholera, the helminthoses, bubonic and pneumonic plague, epidemic cerebrospinal meningitis, acute poliomyelitis, diphtheria, pneumococcus pneumonia, streptococcal affections, influenza (including infections with the Pfeiffer bacillus), filariasis and other diseases. Carriers have been described for tuberculosis, syphilis, and malaria, although the correctness of this has been questioned. In spite of their tendency to chronicity and latency they seem to follow a progressive course unless actively combated. "Contact" or "passive" female carriers certainly are to be found in syphilis and gonorrhea. Instances are known where it is probable that the male has become infected through a female who did not herself contract the disease. The possibility exists that we are sometimes dealing with an analogous case when a syphilitic child is born of a mother free (as is alleged) of demonstrable syphilis. It is likely that carriers exist in certain diseases of debatable etiology (possibly due to the "filterable viruses"), *e. g.*, measles, scarlet fever, mumps, smallpox, chickenpox, encephalitis lethargica and the common cold. Carriers of Vincent's fusiform bacilli and spirochetes undoubtedly exist, and were important in the dissemination of "trench mouth" during the World War.

The carrier is either (1) one who has experienced the disease in question and is convalescent or recovered, or (2) one who has never had the disease himself but has acquired the organisms by *contact*, direct or indirect, with a patient or another carrier. The individuals belonging to the first category are often termed "active" carriers

while to the second category the term "passive" or "contact" carriers is applied. While the distinction is easily drawn in theory, it is not always easy in a given case positively to put the individual down as a passive carrier. The reason for this difficulty lies in the fact that many cases of infection follow such a mild or atypical course that diagnosis by a physician, even if one be called in, is not made unless an epidemic is in progress. Such abortive or ambulant cases are known to occur in some infections and probably occur in all. Hence to designate a person as a passive carrier is hardly more definite than to say that the carrier gives no history of having had the disease. The active and passive carriers may each be subdivided into *temporary* (transient) and *chronic* carriers. The time when a temporary carrier becomes a chronic carrier varies for the different diseases and is determined by clinical experience based on bacteriological findings. For example, "it has been customary to take a period of ten weeks, dating from the commencement of the attack or a relapse, as the limit of what may be called the normal period of residence of *B. typhosus* in the organism which has survived the infection." In the *temporary* carrier the bacilli may be found after that ten-week period, continuing for weeks or months or even a year. After that, by a useful but rather arbitrary decision, they become *chronic* carriers, when the condition may persist for years, continuing through the life of the individual. The contact or passive carrier may at any time contract the disease and manifest all the classical, clinical symptoms. The term "precocious" carrier is applied to one who exhibits the carrier condition during the incubation stage of the infection.

The above paragraph can be summarized by saying that a certain number of persons recovering from many infectious diseases remain carriers after recovery, and that also a certain number of healthy individuals who have come into more or less close contact with a patient (or another carrier) acquire the carrier condition. The reality of the passive contact carrier is strongly brought out by the studies of Avery *et al.*,¹ as apparent in the following table.

Pneumococcus.	Percentage incidence in saliva of 297 healthy non-contacts.	Percentage incidence in saliva of 309 healthy contacts.
Type I	0.33	13.1
Type II	0.00	12.1

¹ Avery, *et al.*, Monograph No. 7, Rockefeller Inst. Med. Res., 1917, pp. 94, et seq.

The various phases and importance of the carrier problem can be understood best by reference to a concrete instance. The case of typhoid fever is selected because this field has been so extensively studied *vd. Garbat*.¹ The carrier discharges the typhoid bacilli either by feces or urine or both feces and urine. Consequently "feces" carriers and "urine" carriers are recognized. The bacilli found in the feces may come from persisting foci in the liver, gall-bladder or intestine proper. Combinations of these sources in the same individual are known. Thirty-two per cent of typhoid patients become *feces* carriers; 28 to 29 per cent temporary and 3 to 4 per cent permanent. Symptoms of gall-bladder trouble, particularly together with a history of typhoid, are suggestive of the carrier condition. "Bile" typhoid carriers are far more frequent in females than in males (60 to 82 per cent females). Six and seven-tenths per cent of all typhoid cases show a bacilluria (are in a sense *urine* carriers) for one to two months after the absence of fever. In only 1.2 per cent of all typhoid cases does the bacilluria continue for two to three months. The persisting foci may be either in the kidney itself (one or both) or in the bladder. The percentage of typhoid carriers in the total population is usually estimated to be under 1, probably about 0.3 to 0.4 per cent. It is naturally difficult to decide with any degree of certainty what proportion of typhoid cases is due to carriers. One estimate (Frosch) has been as low as 5.01 per cent. On the other hand, Simon (p. 81) believes that 40 per cent is a conservative estimate. It is certain that with improving care of water and food supplies, the percentage of cases due to carriers (and hence the relative social menace of the carrier) is rising.

The Carrier Problem in Relation to Dentistry.—If the patient be a carrier, especially of the microorganisms of the respiratory diseases, the dentist is peculiarly exposed to infection or to acquire the carrier condition himself by contact. The respiratory diseases include the exanthemata, whooping cough, pneumonia, epidemic cerebrospinal meningitis, anterior poliomyelitis, influenza, the common cold, and diphtheria. The dentist should remember in this connection that the carrier is recruited from convalescents and individuals who have come in contact with patients or other carriers. Where he is aware that the patient has recently recovered from such a disease or in whose immediate family circle there has been recently or still is a case of such a disease, the dentist should always keep in mind

¹ Typhoid Carriers and Typhoid Immunity, Rockefeller Inst. of Med. Res., Monograph No. 16.

the possibility that the patient may be a carrier. On occasions when one of these infections is epidemic in the community it might be well to postpone all except urgent and emergency treatments because it is a well-established fact, repeatedly confirmed in the experiences at the mobilization camps of the World War, that just antecedent to and accompanying epidemics the number of contact carriers is enormously increased. The observation of Eggebrecht¹ is important. He has found that occasionally typhoid bacilli are eliminated from the mouth for a longer period than from the intestine. In 200 cases of typhoid patients, who had shown symptoms of oral or nasal catarrh, cultures from their expectorations and scrapings from the tonsils, pharynx and tongue, showed typhoid bacilli present 9 times (4.5 per cent). Their feces were free of these bacteria. Eggebrecht has cultivated typhoid bacilli from the mouths of convalescents and also healthy contacts, thus establishing the existence of "mouth" typhoid carriers.

In the above paragraph attention has been called to the danger to which the dentist is exposed from carriers among his patients. The danger, it is to be remembered, is not only to himself but also to his other patients if he himself becomes a carrier. This side of the question is ethically the more important. The intimate relation of the dentist to his patient exposes both of them mutually to infection or to the acquirement of the carrier condition. It is, of course, the moral duty of the dentist to be sure that he does not serve as a carrier of infectious disease to his clientele. This means, if he has any reason to suspect that he is a carrier, that he take steps to determine that point one way or the other. If examination prove that he is one, then he must enforce the most scrupulous precautions upon himself. The particular reasons for such suspicion are: (1) The having passed through one of the diseases to which the carrier condition is not unlikely to be a sequel, and (2) the exposure to a case of such a disease in his immediate family circle.

While the dentist is professionally exposed to the dangers of carriers among his patients for the greatest part only in the case of the so-called respiratory diseases, he himself may be an effective carrier of both the respiratory and intestinal infections (particularly typhoid and paratyphoid fevers and the dysenteries). In the latter category transmission occurs most frequently by the hands which are readily contaminated at defecation or urination. The carrier must exercise the greatest cleanliness. The routine, thorough

¹ München. med. Wehnschr., 1910, **63**, 401.

cleansing and drying of the hands immediately after defecation and urination and before beginning work on each patient will go a long way to prevent the transmission of infectious microorganisms. "Gaehtgens¹ has performed an interesting series of experiments designed to determine the most efficient, and at the same time the least irksome, method of cleansing the hands of typhoid carriers. He smeared the hands and fingers with feces containing typhoid bacilli, and determined the relative disinfectant values of mechanical washing, with and without disinfectants, and followed or not followed by thorough drying. He found that washing with soap and water alone, without subsequent drying of the hands, caused a marked fall in the number of *B. coli* and *B. typhosus*, especially of the former. If, however, the hands were dried thoroughly after the removal of the soap in running water, subsequent cultivation frequently showed a complete disappearance of *B. coli* and *B. typhosus*. For such carriers, however, whose occupation calls for the intimate use of the hands and fingers as occurs in dentistry, "it is advisable to employ an antiseptic after the ordinary washing and drying process (of course an intestinal or urine carrier should never share a towel with anyone else), in order to insure complete removal of any typhoid germs still clinging to the hands. For this purpose Gaehtgens found that the most satisfactory disinfectant was alcohol, either in the form of eau-de-Cologne or spirits of wine."

¹ Quoted from Ledingham and Arkwright, 1912, p. 130.

CHAPTER VIII.

THE CHARACTERISTICS OF INFECTION.

INCUBATION PERIOD.

ONE of the most prominent and earliest recognized peculiarities of an infectious disease is the *incubation period*, a period of latency between the time when the microorganisms must have gained access to the host and the time when symptoms began to manifest themselves. This incubation period is best marked in the case of acute infections or at least of infections with an acute onset. However, it probably is never lacking, no matter how much abbreviated or how unobtrusively the symptoms are initiated.

The reality of an incubation period is distinctly shown by Pasteur's early work on rabies. By repeated passages through rabbits the effectiveness of the virus was so increased that the length of the incubation period was cut from fourteen days to seven days. But continued passages could not lessen this value, which maintained itself as an irreducible minimum. While particular infections tend to have incubation periods of a characteristic average length, they are in the individual clinical case subject to wide variations. In typhoid fever, for example, the duration is from eight to fourteen days, sometimes twenty-three days; in smallpox, usually from five to seven days; in scarlet-fever from one to seven days, oftenest two to four; in measles from seven to eighteen days; oftenest fourteen; in epidemic parotitis (mumps) from one to three weeks; in influenza from one to four days, oftenest three to four days; in diphtheria from two to seven days, oftenest two; in syphilis three weeks to a month usually lapse between exposure and the appearance of the primary lesion or hard chancre; in gonorrhea the incubation period rarely exceeds one week; in rabies the average is from six weeks to two months; and in tetanus usually under ten days.

The varying interplay of many factors accounts for this variability; and the significance of the incubation period is bound up with these factors. In the first place among these factors would be the number of bacteria introduced, their virulency, etc. (See section on How Bacteria Produce Disease.) Contrasted with this would

be that complex of factors connoted by the term "resistance" of the host. Probably very rarely, if ever, under natural conditions, is the number of bacteria initially introduced, of itself and without increase, capable of producing clinical symptoms. If this be true, then the incubation period represents a time during which the microorganisms are increasing in numbers. In experimental syphilis in the rabbit, Chesney and Kemp¹ have shown that the size of the dose of infecting material exerts an appreciable effect upon the length of the incubation period. "Other things being equal, the larger the dose the shorter is the incubation period." For example, when the inoculum was 0.8 cc the average duration of the incubation period was ten days. By decreasing the inoculum to 0.2 cc, the period was lengthened on the average to 17.8 days. If the defensive forces of the host arrest the multiplication of the bacteria and accomplish their destruction before clinical symptoms appear, then the patient and his physician are ignorant of the danger that threatened. Such *abortive* infections are probably far more frequent than usually realized. If the defensive forces prove inadequate at this stage, the bacteria continue to increase until the volume or quantity of damage they inflict on the host calls forth a clinically recognizable reaction. It has also been suggested that this does not represent the whole story of the incubation period. We may be also dealing here with phenomena of an allergic nature. The period of latency is a period during which the host is being *sensitized* to the products of the microorganism in question. The clinical onset is occasioned by, and indicates the success of, this process of sensitization.

The incubation period involves something besides (1) Time for the growth of an effective bacterial mass, and (2) time for sensitization. This is apparent from the fact that no matter how large a quantity of diphtheria toxin or tetanus toxin is introduced into a guinea-pig or mouse, there persists a certain, irreducible minimum period of latency.

Infection is characterized by many changes, morphological and physiological, in the patient. These changes are in part due directly to the damage done by the parasite and in part they represent reactions to the presence of the parasite. Fairly definite, characteristic groups or complexes of these changes (syndromes) are presented by the several infections, *i. e.*, by the several types of

¹ Jour. Exper. Med., 1925, 41, 479.

clinical entities. But no one type presents all the changes and even for a particular case of a given disease, the symptom complex is more often atypical than not.

These morphological and physiological departures from the normal include: (1) Temperature changes; (2) cardiovascular and respiratory changes; (3) changes in the erythrocyte; (4) changes in the leukocyte; (5) changes in the blood plasma; (6) changes in the urine; (7) loss of weight; (8) some histological and cytological changes, *e. g.*, cloudy-swelling, the fatty metamorphoses, amyloid infiltration, necrosis, inflammation, repair, etc. The presence of specific antibodies in increased quantities in the body fluids and the activities of phagocytic cells will, because of their great importance, be considered separately later.

TEMPERATURE CHANGES.

Most infections except those running an extremely low-grade, chronic course are accompanied by departures from the normal in the patient's temperature. These are expressions of a disturbance in the thermo-regulatory mechanism: (*a*) Either thermogenesis is increased without compensatory change in the dissipation of heat or (*b*) the dissipation is reduced without a corresponding decrease in thermogenesis or (*c*) both heat-production and heat-dissipation are involved. These disturbances in turn are ascribable to toxic substances which may be derived from the invading microorganism or from an altered metabolism of the cells of the host. There is no general agreement as to the nature of these substances or as to their pharmacodynamics. During fever the cutaneous vessels dilate and the skin is flushed. The sweat glands may or may not function, resulting in a moist or dry skin. Protein constituents, which actually form a part of the living protoplasm, are more rapidly oxidized than normally. The same process happens to the carbohydrates and fats. In certain febrile disease, *e. g.*, pneumonia, the chlorids may be retained and water-retention is common.

In acute infections, three stages of fever can be distinguished:¹ (1) The *pyrogenetic*, during which the temperature rapidly or slowly rises; (2) the *fastigial*, during which it remains at its maximum; (3) the *defervescent*, during which it returns to normal: if rapidly, then by *crisis*; if slowly, then by *lysis*.

It is also customary to recognize *continued*, *remittent*, and *inter-*

¹ Adami: Principles of Pathology, Philadelphia, 1908, vol. 1.

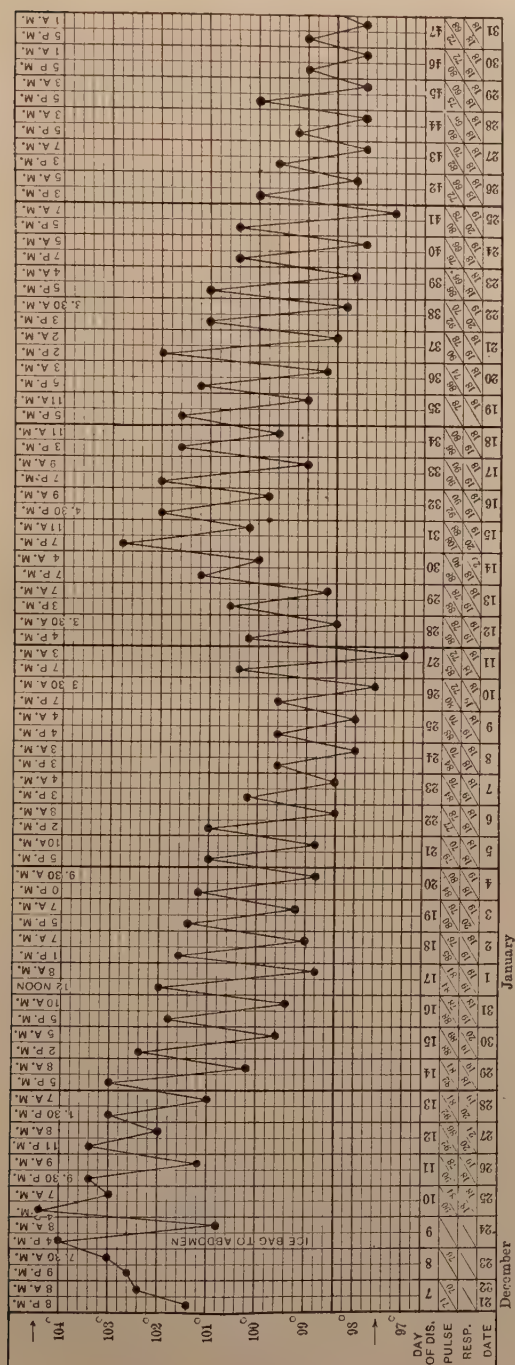


Fig. 33.—Typical chart, mild typhoid with an intercurrent relapse. (Musser.)

mittent and *recurrent* fevers. In the continued fevers as typhoid and pneumonia, the daily temperature changes during the fastigium, although occurring at a higher lever, are little if at all greater than those seen in health. (Figs. 33 and 34.)

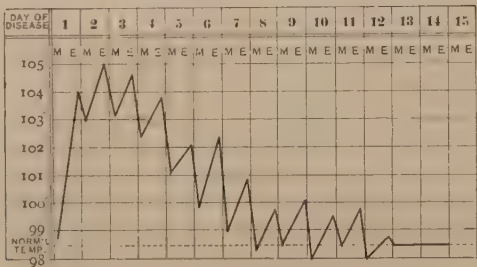


FIG. 34.—Chart of scarlet fever. (Hare.)

In remittent fever, as in pyemia, suppuration and tuberculosis with secondary infection, the daily temperature changes extend over several degrees. The temperature curve, while continuing above the normal is very variable. In intermittent and recurrent (or relapsing) fever there is a succession of febrile attacks, each characterized by pyrogenetic, fastigial and defervescent states. The several attacks are separated from each other by an interval, varying from hours to days, during which the temperature is normal. Malaria, relapsing fever proper and Malta fever illustrate this variety. (Fig. 35).

The clinician, as indicated in the following table, recognizes certain differences in the degree of body temperature.

Subnormal	Below 98.3° F.	
Normal	98.6° F.	37.0° C.
Hypernormal	98.8° to 100.0° F.	37.2° to 38.0° C.
Light fever	100.4° to 101.3° F.	38.0° to 38.5° C.
Moderate fever	101.3° to 103.1° F.	38.5° to 39.5° C.
High fever	103.1° to 104.9° F.	39.5° to 40.5° C.
High fever (evening temperature)	104.9° to 105.8° F.	40.5° to 41.0° C.
Hyperpyrexia, over	105.8° F.	41.5° to 42.0° C.

The temperature that is immediately fatal to mammals (47° C. or 117° F.) is exactly the same as the coagulating temperature of the lowest coagulable protein of the nerve cells. This degree is probably never reached in man. The application of a much lower temperature (even 42° C. or 108° F.) will cause the coagulation of these proteins according to the following principle; all proteins coagulate at less than their ordinary coagulating point if the heating be con-

tinued for a long time. Breitmann¹ has attempted to develop a mathematical expression to indicate the severity of infection in a given case. The calculation is based on the intensity and duration

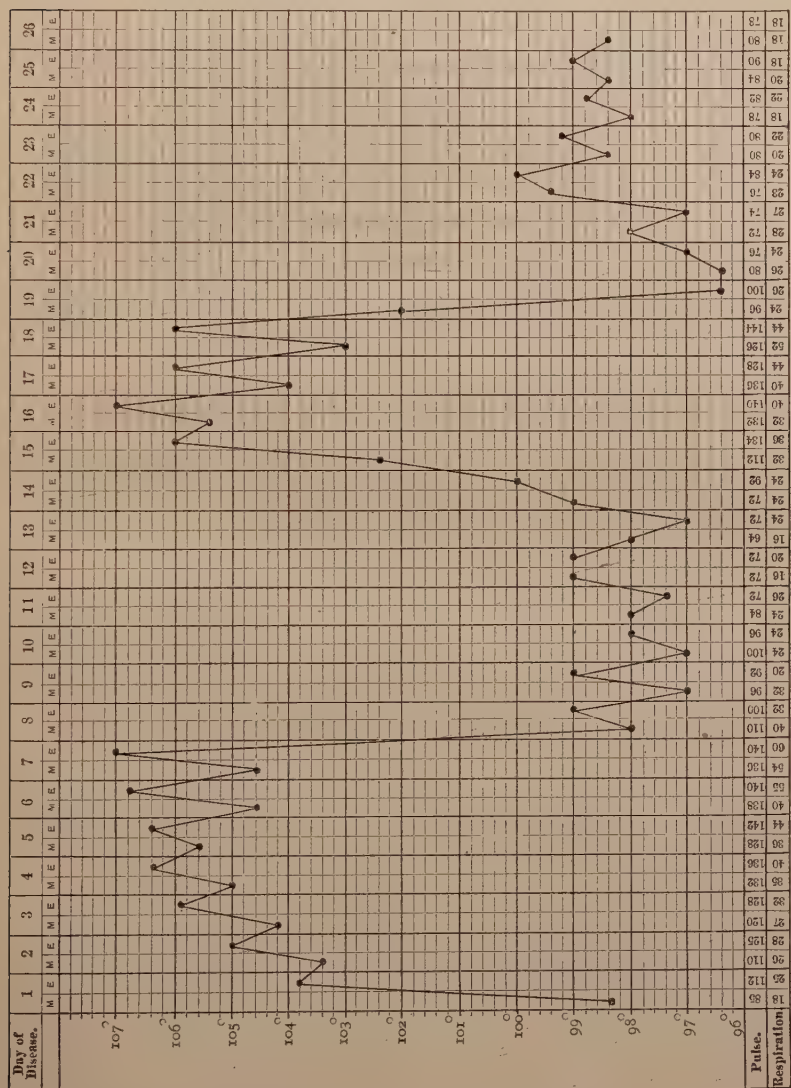


Fig. 35.—Typical case of relapsing fever terminating in recovery. One relapse with slight postcritical rise in temperature. (Hare.)

of fever, and the result is given in temperature-time units. It has long been recognized that mild infections are accompanied by mild

¹ Centralbl. f. Bakteriologie, 1923, 90, 301.

degrees of fever while in general the temperature rises higher in the severer infections. This parallelism is not invariably to be seen; and in fact in weak or debilitated individuals or in the presence of an overwhelming invasion, the fever may be mild or it may even be replaced by subnormal temperatures.

The suggestion has frequently been made that fever is an adaptive reaction which benefits the host by subjecting the parasite to a temperature above its optimum. It is very difficult to get convincing evidence on this. The work of Schwarz¹ is against this interpretation. In studying the influence of temperature on the bactericidal power of the blood he found that a rise in temperature injures

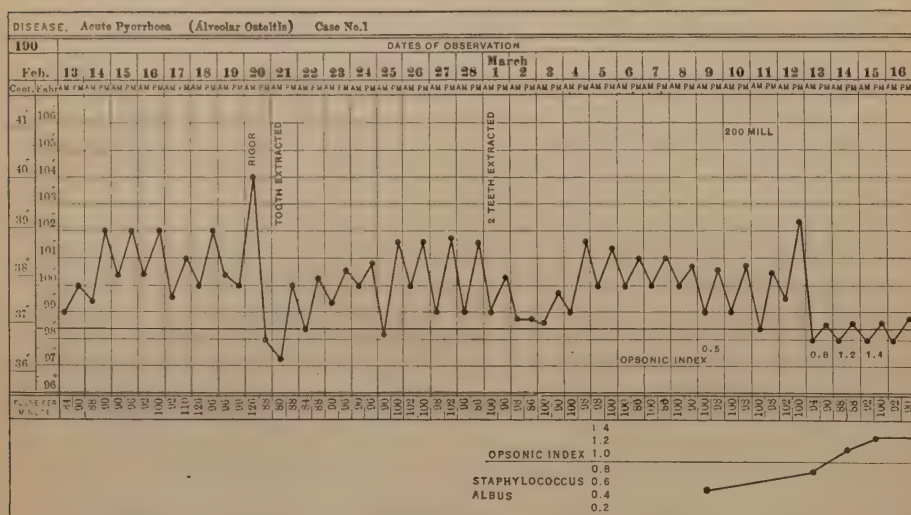


FIG. 36.—Case I, N. B. *a*, drop in temperature following the extraction of the teeth on February 21 and on March 1; *b*, the drop in temperature and its maintenance at normal following the injection of 200,000,000 bacteria in an autogenous staphylococcal vaccin on March 12. (Goadby: Brit. Med. Jour., August 22, 1908.)

the blood more than the bacteria. He also observed that micro-organisms may be more resistant to higher temperatures in the body than *in vitro*. Streptococci isolated from a patient with a temperature of 41° C, could not be cultivated artificially above 40° C.

Experimentally it has been demonstrated that hypernormal temperatures increase the resistance of animals to infections. Moderate over-heating increases the production of agglutinins, bacteriolysins and antitoxins. Rolly² and Rolly and Meltzer³ have

¹ Deutsch. med. Wchnschr., 1924, 50, 754.

² Ibid., 1911, 37, 2121, 2186.

³ Deutsch. Arch. f. klin. Med., 1908, 94, 335.

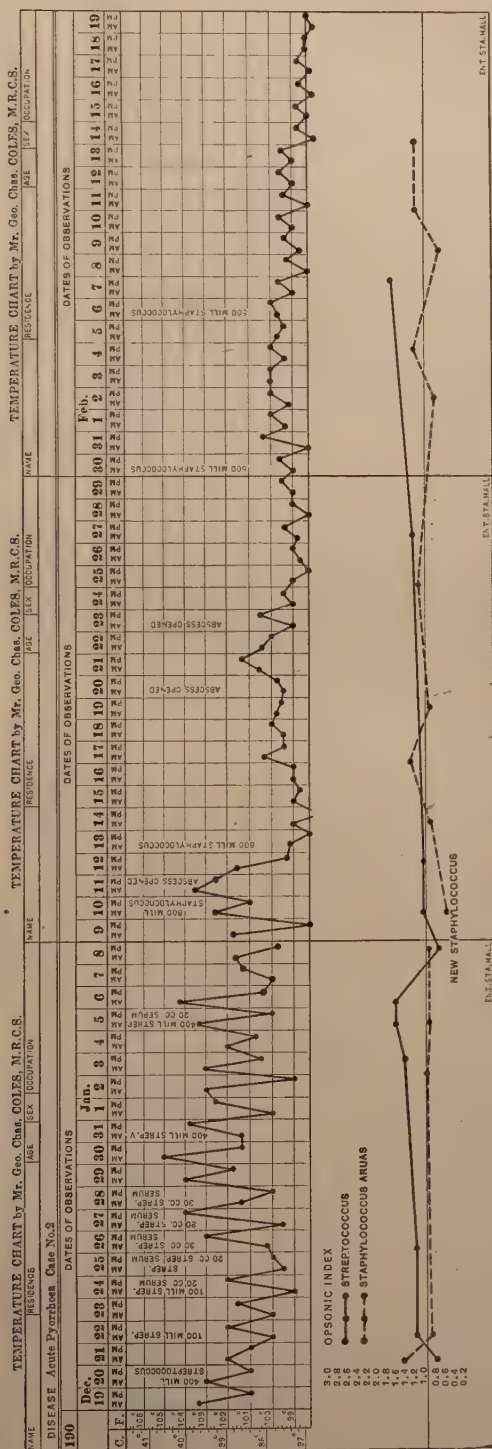


Fig. 37.—Case II. Note at the left of the chart the septicæmic type of temperature, gradually returning to normal in the course of treatment which included the administration of autogenous vaccin. (Goadby: Brit. Med. Jour., August 22, 1908.)

reported that temperatures of 39.5° to 40° C. favor the production of antibodies while above this unfavorable effects appear.

It is well known that elevation of temperature within certain limits accelerates chemical and physico-chemical processes. The rate of union of antibody with antigen and of phagocytosis forms no exception to this generalization; but the benefit which accrues to the febrile host in this way has never been quantitatively estimated.

A peculiar temperature reaction is that of chill. The clinical course of many infections frequently begin this way. Although in this condition the patient experiences a sensation of cold, the body temperature is above normal.

Subnormal temperatures also occur in infections, but are much rarer and of much graver import than the elevations ordinarily observed.

Temperature changes during the common oral infections are usually absent, of trivial extent or of brief duration. These considerations account for the little attention usually given to this phase of the subject by the dentist. However, it is well known that oral infections, especially during acute exacerbations of periapical and periodontal disease, are accompanied by fever, often of some intensity. This is illustrated by reference to two cases reported by Goadby.¹ (Figs. 36 and 37.)

In Case I, attention should be directed to the temporary drop in temperature following the extraction of two teeth on the first of March and to the permanent return to normal following the administration of an autogenous vaccin on the twelfth of March. In Case II, note should be made of the rapid, though temporary, return to normal following the opening of an abscess on March 11th.

Egger² reports a case of a woman with much periodontal infection showing a temperature of 37.6° C. to 37.8° C. during the puerperium. The temperature returned suddenly and permanently to normal following the extraction of the involved teeth.

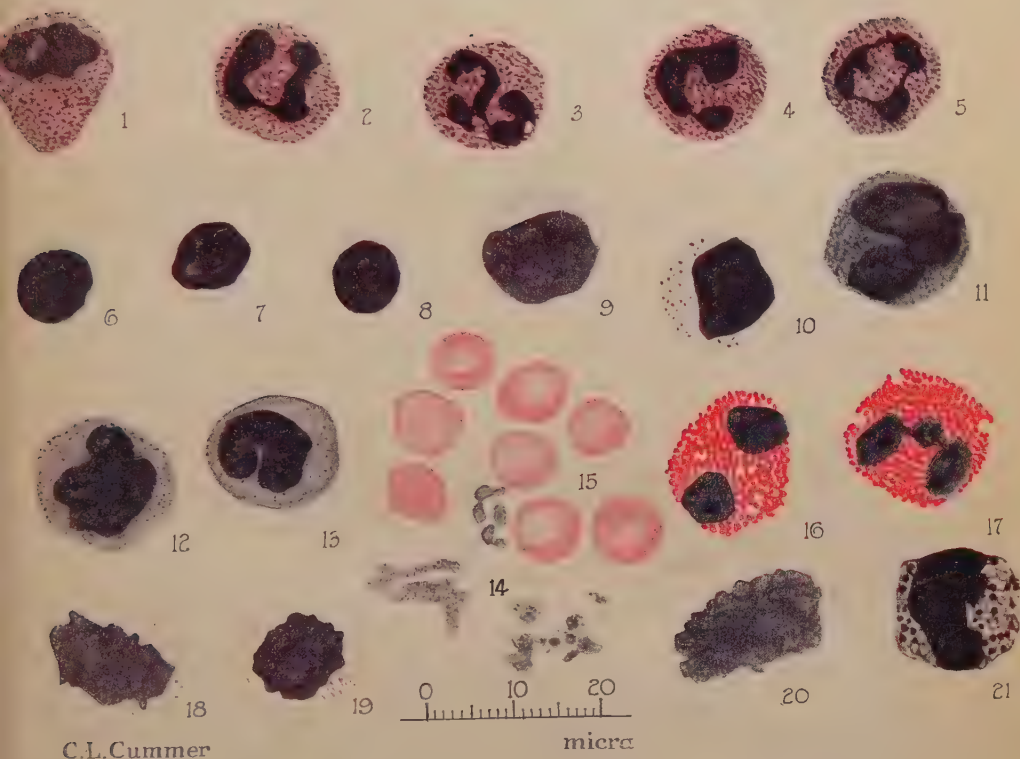
VASOMOTOR, CARDIOVASCULAR AND RESPIRATORY CHANGES.

Accompanying fever, there is usually to be noted an increased rate of the pulse and of the respiration. A rise of 1° F. often entails an increase of 7 to 9 beats per minute. Blood-pressure may be reduced as in tuberculosis and typhoid.

¹ Brit. Med. Jour., August 22, 1908.

² Schweiz. Monatschr. f. Zahnk., 1923, **33**, 686.

PLATE II



C.L.Cummer

Types of Cells Found in Normal Blood. (Cummer.)

All cells drawn with the same magnification and outlined with the camera lucida for the purpose of comparing sizes. Each division of the scale at the bottom of the plate represents one micron. Magnification, 1150.

Stained with Wright's stain. Nos. 1 to 5, inclusive, polymorphonuclear neutrophilic leukocytes; 6, 7 and 8, lymphocytes; 9, 10, large mononuclear leukocytes; 11, 12, 13, transitional leukocytes; 14, a group of platelets; 15, a group of red blood cells; 16, 17, polymorphonuclear eosinophilic leukocytes; 18, 19, 20, "basket cells," degenerated leukocytes; 21, "mast-cell," basophilic leukocyte.

Experimental infections with the pneumococcus, the diphtheria bacillus and *Pseudomonas aeruginosa* all exhibit great falls in pressure attributable to paralysis of the vasomotor centers or to injury to the myocardium. Price¹ reports a case where the blood-pressure which had been about 225 for some time rapidly dropped to 125 mm. Hg. and remained there, after the opening and curettage of a dental cyst.

CHANGES IN THE BLOOD.

Morphological.—*Erythrocytes.*—The changes in the erythrocyte during infection involve both its number and its content of hemoglobin. The word anemia is a rather loose term covering both these aspects when the change is a diminution. Many of the so-called secondary anemias are infectious in origin; as in tuberculosis, syphilis and malaria. In acute diseases during fever the destruction of the erythrocytes is masked by the relative concentration of the blood. With defervescence there often occurs a sharp fall in the number of red cells per unit volume (cubic millimeter). During typhoid fever the erythrocyte count may drop from about 5,000,000 to a little over 2,000,000, *i. e.*, a drop of over 50 per cent. Similar reductions occur in smallpox, acute articular rheumatism, and severe cases of septicemia.

The erythrocytes during infection may show structural changes. Normoblasts and megaloblasts may appear in pneumonia. Poikilocytosis may be seen.

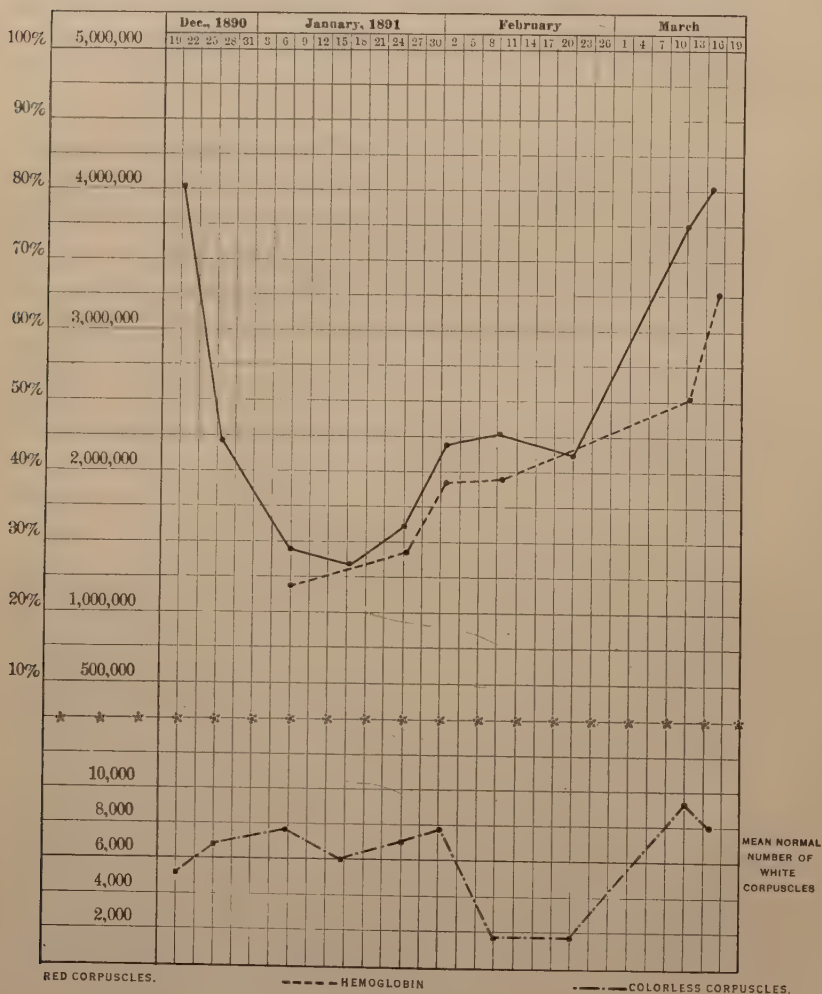
The hemoglobin in typhoid, for instance, shows a gradual decline during the fever, dropping not infrequently to 50 per cent. The hemoglobin percentage suffers more than the count of corpuscles and is slower in recovery. Low percentages are the rule in septicemia, syphilis, malaria, and hook-worm infection.

The accompanying chart shows graphically the decrease in the number of erythrocytes and in the hemoglobin percentage with recovery, during convalescence. (Fig. 38.)

Leukocytes.—The changes in the leukocytes during infection may affect both their number per unit volume (cubic millimeter) and the ratios existing between the several types of these cells. The term leukocyte in this connection refers to any of the white cells of the blood. Various classifications of these cells have been proposed but the one most satisfactory at present uses as its criteria the shape of the nucleus and the presence or absence of different kinds of cytoplasmic granulations. (Plate II.)

¹ Dental Infections, 1923, 2, 111.

Polymorphonuclear.—The nucleus exhibits two or more lobes, whose connections may be so attenuated that they easily escape notice (hence the incorrect term “polynuclear”).



3. *Basophilic* (Synonym: Mast cell).—Coarse, bluish, cytoplasmic granules.

Mononuclear.—The nucleus is not lobed, no chance for regarding the cell as bi- or multinucleate; when stained by ordinary methods, *e. g.*, Jenner's or Wright's, the cytoplasm is free of granules.

1. *Lymphocyte* or small mononuclear.—Nucleus regularly circular in outline, very small zone of peripheral cytoplasm; smallest of all the leukocytes.

2. *Large mononuclear* (tentatively included here are the endothelial leukocyte of Mallory and the transitional cell).—The largest of all the leukocytes; nucleus circular, oval or horse-shoe-shaped; peripheral cytoplasm abundant.

The number of all the leukocytes in a cubic millimeter of peripheral blood normally is about 5000 to 6000. This is slightly increased soon after meals, the so-called postprandial hyperleukocytosis. As a general rule a count of 10,000 or more is regarded as abnormal. A hyperleukocytosis is commonly encountered in pneumococcal, staphylococcal, and streptococcal infections. For example, in pneumonia most counts range from 10,000 to 30,000, but may in exceptional cases rise to 100,000. In osteomyelitis where staphylococcal infection is often present counts ranging from 18,000 to 30,000 occur. In puerperal septicemia where streptococci are the chief aggressor, counts around 20,000 are not uncommon and approximations to 100,000 are not unknown. Acute appendicitis shows a well-marked hyperleukocytosis. While most of the counts remain under 20,000, doubling or tripling of that value occurs. With the disappearance of the infection the leukocyte count returns to normal. The accompanying Fig. 39 shows this. The temperature curve may be taken as indicative of the patient's general condition.

On the contrary, in typhoid fever, influenza and uncomplicated tuberculosis there is no increase in the number of leukocytes and there may even be a decrease (a hypoleukocytosis or leukopenia). In typhoid for example, one often sees a slight, transient, initial hyperleukocytosis, rapidly succeeded by a drop below normal (leukopenia). See lower part of Fig. 38.

Under normal conditions, the relative number of the several kinds of leukocytes remains fairly constant. The polymorphonuclear neutrophils comprise about 62 to 70 per cent of all leukocytes in the normal circulating blood; the percentage of eosinophils varies from 0.5 to 4; the percentage of basophils varies from 0.025 to 0.9 per cent. The lymphocytes comprise about 20 to 30 per cent, and the large mononuclears comprise about 4 to 8 per cent. These

percentages vary significantly in many infections. The following table which is adapted from one in Simon's *Clinical Diagnosis* (10th ed., 1922, p. 55), gives the most important deviations from

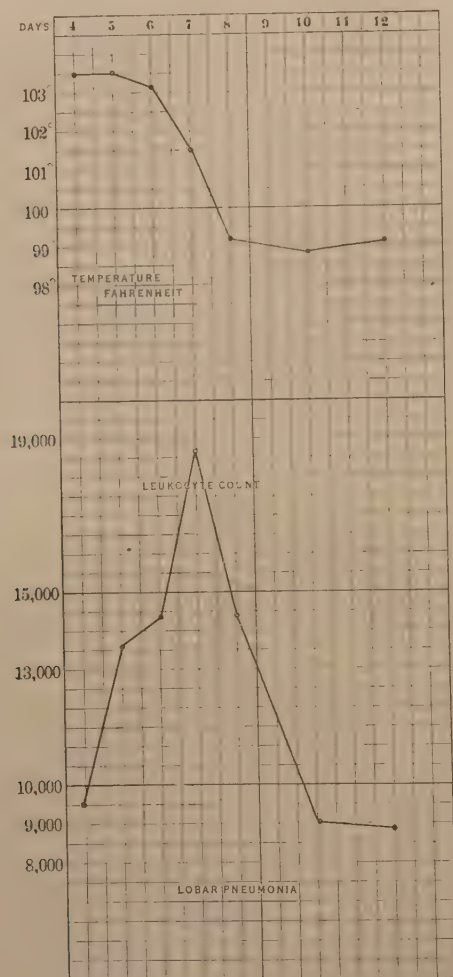


FIG. 39.

the normal leukocytic formula in some of the more common infections. The differential count which reveals these deviations should never be omitted in any morphological examination of the blood.

Neutrophilic hyperleukocytosis.	Neutrophilic leukopenia.	Lymphocytosis.	Lymphopenia hypoeosinophilia.	Hypereosinophilia.
All pure infections with: Streptococci Staphylococci Pneumococci Meningococci Catarrhal micrococci and colon bacilli Common wound infections Pneumonia Erysipelas Meningitis Peritonitis Appendicitis Tonsillitis Salpingitis Puerperal endometritis Diphtheria Acute rheumatism Empyema Various abscesses	Typhoid fever Paratyphoid fevers Measles Uncomplicated tuberculosis Influenza Vincent's infection (Tarnow)	Whooping-cough Congenital syphilis Measles Typhoid fever Tuberculosis Influenza Vincent's infection (Tarnow)	Various diseases given in the first column	Scarlatina. Various skin diseases. Hook-worm diseases. Trichinellosis. Gonorrhea. Certain cases of tuberculosis during convalescence from pyogenic diseases mentioned in first column. Vincent's infection (Peter, 1 case).

The leukocytic response in infections is largely defensive or protective in function. It has been frequently pointed out that a steady rise in the leukocyte count during the course of a disease is usually of favorable import. This is illustrated by the following table, resuming the observations in a particular case of pneumonia.¹

Day of disease.	Leukocyte count.	Poly-morpho-nuclears, per cent.	Lympho-cytes, per cent.	Large mono-nuclears, per cent.	Transi-tionals, per cent.	Eosino-phils, per cent.	Tempera-ture, °F.
Fourth	9,400	93	4	3	...	...	103.6
Fifth	9,400	96	4				
	13,600	87	4	7	2	...	103.6
Sixth	14,400	88	5	5	2	...	103.2
	14,500	86	13	...	1	...	103.6
Seventh	18,700	94	6	...	...	...	101.4
	20,000	86	4	5	5	...	100.2
Eighth	14,400	84	6	5	4	1	99.2
	12,000	85	3	6	2	4	100.0
Tenth	9,000	78	6	...	13	3	98.8
Twelfth	8,800	68	10	16	4	2	99.2

¹ Avery, Chickering, Cole and Dochez: Monograph No. 7, Rockefeller Inst. Med. Res., 1917, p. 40.

This is not an exceptional case but rather the rule as appears from the subjoined table.¹

THE RELATION OF LEUKOCYTES TO MORTALITY IN LOBAR PNEUMONIA.

Leukocytes.	No. of cases.	Mortality, per cent.
Under 10,000	29	65.5
10,000 to 20,000	143	23.7
20,000 to 30,000	177	18.0
30,000 to 40,000	76	14.4
40,000 to 50,000	29	24.1
Above 50,000	9	11.0
Total	463	

Notice is particularly directed to the high mortality when the leukocytes are under 10,000 in number. This is confirmatory of an observation made by Becker² who reported 83 deaths out of a total of 90 pneumonia cases, with leukocytes under 10,000.

The Morphology and Hemoglobin Content of the Blood in Oral Infections.—Isolated attempts on relatively small series have been made to discover some characteristic of the blood-picture in oral infection. This has been done with the idea of determining the nature and the extent of systemic involvement. As yet the data are too scant to justify any generalization. Nothing distinctive has as yet revealed itself.

What is known of the morphological changes in the blood during Vincent's infection, is described in the consideration of that condition.

Bunting³ reported that the number of circulating basophilic leukocytes is increased in chronic inflammations of the accessory nasal sinuses.

In 1904, Cabot in the fifth edition of his well-known *Clinical Examination of the Blood*, incidentally remarked that even a "gumboil" raised the white cells to 27,000 in one case. Such hyperleukocytoses may occur more frequently than realized in acute stages of oral infections.

About the beginning of the twentieth century William Hunter layed much stress upon oral sepsis as a cause of anemia, particularly pernicious anemia.

Goadby⁴ published the results of some blood examinations on patients, presenting no other discoverable cause than pyorrhea alveolaris.

¹ Avery *et al.*: *Ibid.*, p. 39.

² *Deutsch. med. Wchnschr.*, 1900, p. 558.

³ *Physiol. Rev.*, 1922, 2, 505.

⁴ *Brit. Med. Jour.*, September 9, 1905, p. 562.

Case.	Date.	Hb.	R.B.C.	W.B.C.	Ratio.	C. index.	Remarks.
Miss 7.	Jan. 22, 1904	74	3,850,000	10,000	385:1	0.9	Sequestrum removed.
	Sept. 30, 1904	70	3,900,000	9,000	437:1	0.8	
	Oct. 28, 1904	80	4,000,000	8,000	500:1	0.9	
	Feb. 6, 1905	70	3,930,000	10,000	393:1		
	May 1, 1905	77	4,760,000	10,000	476:1	0.7	1 wk. after vaccin.
	Jan. 5, 1905	80	3,860,000	8,000	482:1	0.9	1 wk. after vaccin.
Con. B.	Nov. 18, 1904	78	3,600,000	9,000	400:1	0.9	
	Dec. 17, 1904	92	4,440,000	8,000	551:1	1.0	
Miss B.		72	3,200,000	9,000	355:1	0.9	
Miss R.	Nov. 17, 1904	70	3,800,000	9,000	423:1	0.8	
Miss P.		80	3,500,000	8,000	437:1	1.0	
Miss F.		75	3,550,000	10,000	355:1	0.9	

Hb = hemoglobin; R. B. C. = red blood cells; W. B. C. = white blood cells; C. index = color index.

Two years later Goadby¹ stated that of the various general symptoms associated with pyorrhea alveolaris the commonest are anemia of a secondary nature, a hemoglobin of from 60 to 70 and a moderate leukocytosis. The erythrocyte count is about 3,000,000.

Goadby² has found in the earlier stages of pyorrhea alveolaris a leukopenia as the chief change in the blood. This gradually gives way to a hyperleukocytosis. In chronic gingivitis and stomatitis (p. 23) the blood usually shows a considerable hyperleukocytosis.

Simms³ made blood counts in 12 patients with pyorrhea alveolaris. He found a mild anemia in all but 1.

Hartzell, Henrici and Leonard⁴ made red cell counts and hemoglobin determinations in one case of pernicious anemia.

	May 23.	May 29.	June 10.	June 16.	June 25.
Erythrocytes	2,700,000	2,400,000	2,630,000	1,800,000	2,500,000
Hemoglobin	57 per cent	63 per cent	61 per cent	55 per cent	65 per cent

What is of special interest is that the noticeable drop in red cells and hemoglobin on June 16th, occurred coincidentally with the development of a dental abscess. The extraction of the tooth was followed by a rise in red cell count and hemoglobin to the highest values noted during the care of the case.

Logan⁵ reported the blood findings in 162 cases of chronic oral

¹ Lancet, March 9, 1907, p. 633.

² Diseases of the Gums and Oral Mucous Membranes, Oxford Med. Pub., 1923, p. 344.

³ Trans. Odont. Soc. Great Britain, 1907, **39**, 164.

⁴ Bull. Nat. Dent. Assn., October, 1914, **1**, 52.

⁵ Dent. Items of Interest, 1915, **37**, 912.

infection, of which 110 were cases of advanced pyorrhea alveolaris. The remaining 52 presented chronic periapical infection (18 of these also had pyorrhea alveolaris). In 100 of the 162 cases significant changes in the blood were found. Neither the number of erythrocytes nor the hemoglobin percentage were materially affected, *i. e.*, anemia is not the rule. Leukopenia was more constant than hyperleukocytosis in such cases of pyorrhea alveolaris as presented blood changes. Leukopenia was more frequently encountered in those cases where the pyorrhetic pockets did not involve the root-ends and where coincident periapical infection was absent. A hyperleukocytosis in the cases of pyorrhea alveolaris was more frequently observed when the pockets almost or quite involved the root-ends. A hyperleukocytosis was present in 47 of the 52 cases of periapical infections.

Roddy, Funk and Kramer¹ made red cell counts and hemoglobin determinations in 40 early cases of pyorrhea alveolaris. Thirty of these showed some degree of anemia; in 6 the red cell count was 3,000,000 or slightly less and in 12 the hemoglobin (Dare apparatus) was under 75. In 4 of the early cases there was a great increase of the platelets above normal.

Twelve advanced cases were examined and all showed some anemia. The average departure from the normal was greater than in the less advanced cases.

Hecker² gave the following values as characteristic for pyorrhea alveolaris; neutrophilic polymorphonuclears 46 per cent; large lymphocytes 40 to 60 per cent; and small lymphocytes 3 to 5 per cent. One is tempted to suspect that the adjectives "large" and "small" have been typographically reversed. At any rate, Hecker observed a decrease in the number of neutrophils and a lymphocytosis.

Schuhman³ reported a case in which the differential count is given before and after ordinary prophylactic treatment.

	Neutrophils, per cent.	Lymphocytes, per cent.	Large mononuclears, per cent.	Eosinophils, per cent.
Before	58	37	3	2
After	68	26	5	1

This improvement was recorded in spite of the fact that the patient presented 8 teeth with radiolucent areas, which had received no attention.

¹ New York Med. Jour., 1916, **104**, 433.

² Dent. Cosmos, 1912, **54**, 1354.

³ Dent. Summary, 1916, **39**, 492.

Crance¹ reviewed the blood examinations of 200 cases of chronic focal infection. One hundred and seventy-nine presented definite dental infection. Of these 118 showed definite periapical involvement, either with or without pyorrhea alveolaris. One hundred and fifty-seven of the 200 cases showed definite infection in the tonsils and 151 cases had enlarged submaxillary nodes.

The average hemoglobin percentage was 75.8.

The number of red cells was apparently but slightly affected. Only 5.5 per cent of all cases gave a count below 4,000,000.

The results of the white cell count (total) are tabulated below.

	Per cent of cases.
6000 to 6800	29.0
5000 to 6000	27.5
4000 to 5000	4.5
3000 to 4000	1.5
1000 to 2000	0.5

In brief, 63 per cent of the cases showed a definite leukopenia.

Differential counts were made in nearly all cases but showed very little deviation from normal.

In discussing the bearing of his work, Crance emphasizes that a leukopenia is evidence of lowered body resistance to infection.

Daland² reported a study of the leukocytes in 100 cases of chronic periapical infection in adults. In order to simplify the problem he excluded all cases complicated by a focus of infection elsewhere. Leukopenia was present in 54 cases (assuming that 5000 leukocytes per cubic millimeter, or less, represents a leukopenia). In a control series of 100 cases of chronic disease in adults without discernible focal infection, only 6 cases of leukopenia were found. Daland was convinced that a lymphocytosis (small cell) with a corresponding decrease in the polymorphonuclear (neutrophils) cells is an important diagnostic sign of periapical infection. (See also Egger, *infra*, for confirmation.) A coëxistant leukopenia enhances the value of this sign. Lymphocytosis occurred in only 2 of 100 cases of chronic disease without focal infection. The lymphocytosis of focal infection usually disappears in from five to eight weeks after the removal of all foci. Lymphocytosis persisting longer than this after the removal of periapical infection, usually indicates the presence of an undiscovered focus. When the percentage of lymphocytes is increased with leukopenia, the absolute number of lymphocytes may be either slightly increased or decreased. The essential change is a decrease in the polymorphonuclear neutrophils.

¹ Med. Record., October 8, 1921.

² Jour. Am. Med. Assn., 1921, **77**, 1308.

Haden¹ studied the white cell count and the differential count of 200 individuals. These patients were distributed into five groups: I, With definite roentgenographic evidence of periapical involvement; II, with no roentgenographic evidence; III, with periapical involvement and systemic disease of focal origin; IV, with no evidence of systemic disease of focal origin (17 showed radiolucent areas, 28 had no pulpless teeth and 18 had pulpless teeth without radiolucency); V, with systemic disease of focal origin which was reproduced in rabbits by bacteria isolated from periapical foci.

It was found that patients with periapical infection show a slightly higher total white cell count than those without such infection. The difference is somewhat more marked in those patients exhibiting systemic disease of focal origin. The increase is for the most part in the neutrophils although all types of leukocytes show some increase.

Patients with chronic periapical infection do not typically show a lymphocytosis.

Price² gives in tabular form the results of the routine blood examination of 7 patients. All had dental infections of long standing with systemic involvements, in part attributed by Price to the oral foci. Nothing worth remarking appears in the total red and white cell counts or in the hemoglobin percentage. The neutrophils are reduced relatively while the lymphocytes are increased.

Rickert and Palmerlee³ on the basis of many blood counts believe that patients suffering from low-grade infections do have total white counts slightly above the normal. However, other factors and foci, not of dental origin, are likewise responsible for such increases, so that this does not appear to be a reliable means of differentiating dental foci of infection from other foci. These authors refer to the work of E. F. Lewis, who advances the view that a marked basophilia is present in cases of oral infectious foci. This condition would be a valuable pathognomic aid because the other diseases exhibiting a basophilia, such as cancer, actinomycosis, various skin diseases, splenomyelogenous leukemia, chlorosis and septic bone disease, may rather easily be eliminated. Rickert and Palmerlee investigated Lewis' contention and evidently believe that with the evidence available it would be premature to consider it either as an established fact or even as an important aid in determining the culpability of oral infections.

¹ Jour. Am. Dent. Assn., 1923, **10**, 596.

² Dent. Infect., 1923, **1**, 239.

³ Jour. Am. Dent. Assn., 1924, **11**, 783.

It should not be forgotten that non-oral conditions characterized by changes in the blood-picture may at times have oral manifestations. In such cases the mistake must not be made of attributing the blood changes to the oral lesions. One must not forget, however, the possibility of the existence of a vicious circle. A glossitis has long been associated with pernicious anemia (See Hunter, *supra*) and Alikhan¹ discusses the relation of gangrenous stomatitis to leukemia.

SUMMARY.—It is obvious that various observers have obtained very different results in the study of the blood-picture associated with oral infections. These differences are not necessarily mutually contradictory. Different stages in the infection and different degrees of intensity of the infection will naturally manifest themselves differently. It may also have been a question of infection with different organisms. It appears that anemia, if discernible is rarely marked. In acute infections a slight hyperleukocytosis and a slight increase in the percentage of neutrophils may show themselves. When the condition is chronic and the patient is not reacting satisfactorily, a triad of symptoms may be found, none of them as a rule of more than moderate intensity; a leukopenia, a decrease in the percentage of neutrophils, and either a relative or an absolute lymphocytosis. When the condition is chronic but the patient is reacting satisfactorily, no significant change in the blood-picture appears.

This is a tentative effort to harmonize the various findings which superficially at least are so divergent. We are in need of many records of blood-counts before, during and after dental treatments or extractions in one and the same case, as for example Egger² has done. The patient, with multiple oral infection, presented the following blood-counts before and after dental treatment:

	W.B.C.	Neutrophils, per cent.	Eosinophils, per cent.	Lymphocytes, per cent.	Mast cells, per cent.	Transitionals, per cent.
Before : . .	6600	47	3	43	2.0	5.0
After . . . :	7200	69	1	26	0.5	3.5

One fact of importance to remember is that the data presented above definitely prove the reality of systemic involvement in at least many cases of oral infection. A significant departure from the normal in the blood-picture is *prima facie* evidence of some degree

¹ Rev. méd. de la Suisse romande, 1921, **41**, 785. See also Packard and Flood, 1920, Am. Jour. Med. Sci., **160**, 883; and Becks, 1924, Ergbn. d. ges. Znhhlk., **7**, 1.

² Schweiz. Monatschr. f. Znhhlk., 1923, **33**, 686.

of systemic involvement. In such a case the infection, oral or elsewhere, can no longer be rightly regarded as purely or strictly local.

Chemical and Physico-chemical Changes.—Many chemical and physico-chemical changes and readjustments occur in the blood plasma during infection. None of them can be taken to be of invariable occurrence or as distinctive. The organs or parts affected, their condition before the infection and the extent to which they are involved, would all enter into the determination of the changes demonstrable in the blood plasma. Reference to only a few illustrative instances will suffice for our present purpose.¹ Apparently a slight retention of creatinine (between 3 and 4 mg. per 100 cc of blood) occurs in syphilis and sometimes in fevers. Hyperglycemias are occasionally found in infections; and comparatively low blood sugars (from 0.6 to 0.09 instead of the normal 0.09 to 0.12 per cent) have been reported in cases of tuberculosis of the adrenal gland (Addison's disease). Kipp² found in pneumonia a primary hypocholesterolemia. This subnormal value was the more marked, the severer the infection. During convalescence a hypercholesterolemia appeared, giving way eventually to normal values. Marie³ reports a hypercholesterinemia appearing in the horse during active antidiphtheritic immunization. Alterations in the quantitative relationships of the blood serum proteins are known in syphilis and pneumonia.

Condition.	No. of cases.	Albumin, per cent.	Globulin, per cent.	Total protein, per cent.	Globulin to total protein, per cent.
Normal subjects	22	5.6	1.9	7.5	22.5
Syphilis	19	5.0	2.5	7.5	34.0
Pneumonia	8	3.7	2.5	6.2	40.0

Cooke and Whipple⁴ note in many acute infections in man, *e. g.*, septicemia, peritonitis and pneumonia, a definite rise in the non-protein nitrogen and urea nitrogen of the blood. Some cases show a very great rise above normal (over 100 mg. of non-protein nitrogen per 100 cc of blood).

The normal fibrinogen limits may be given as 0.3 to 0.6 per cent with an average of 0.5 per cent per 100 cc of plasma. In pneumonia and septicemia fibrinogen is much above normal, reaching 0.9 per cent. In the general cachexia of tuberculosis on the other hand it may be quite low, 0.1 per cent. The fibrin content of the blood⁵

¹ Myers: *Physiol. Rev.*, 1924, **4**, 274.

² *Jour. Biol. Chem.*, 1920, **44**, 215.

³ *Am. de l'Inst. Pasteur*, 1924, **38**, 495.

⁴ *Jour. Exp. Med.*, 1918, **28**, 223.

⁵ Pfeiffer: *Ztschr. f. klin. Med.*, 1897, **33**, 214; *Centralbl. f. inn. Med.*, 1898, **19**, 1.

is increased in diseases with a hyperleukocytosis, *e. g.*, pneumonia, rheumatism, erysipelas and scarlet fever. In pneumonia the coagulation of the blood is remarkably rapid; in acute articular rheumatism it is slower than usual and this latter condition is also often noticed in appendicitis. Septicemia characteristically exhibits a decreased coagulability.

Peptolytic enzymes are noticeably increased in most infectious diseases where the reaction between the body defenses and in the invading parasite occurs in the blood stream.¹ Beaton² noted an increase in the antitryptic power of the blood in most infections.

The chemical reaction of the blood (its hydrogen-ion concentration) varies from the normal in various infections; and fluctuations in the alkaline reserve are probably of more frequent occurrence.

De Niord and Bixby³ found in 21 cases of focal infection 3.2 to 4.5 mg. of uric acid per 100 cc of blood. The normal values are 1.5 to 2.5 mg. per 100 cc. They recognize that a hyperuricacidemia may occur in other conditions than focal infection, including chronic periapical infections. They mention leukemia, primary anemias, cachexias from whatever cause, and massive doses of roentgen-ray or radium. To this list should be added⁴ nephritis, gout, pneumonia, malignancy, arthritis, lead and mercury poisoning, methanol poisoning and the toxemias of pregnancy. On the contrary certain drugs, notably the salicylates and 2-phenylquinolin 4-carboxylic acid (atophan) are known to lower the uric acid content of the blood in gout and possibly under all circumstances. Schamberg and Brown⁵ also report high values for blood uric acid in a variety of skin diseases, *e. g.*, eczema (3.66 mg.), pruritus generalisata (3.78), pruritus localisata (3.58), psoriasis (3.19), acne rosacea (2.9), acne (2.6) and urticaria (3.3). It is obvious that before a hyperuricacidemia could be taken as evidence of the presence of focal infection it would be necessary to eliminate all of the various conditions mentioned above. It is of course possible that both focal infection and one or more of these other conditions could coexist. These considerations, even in case DeNiord's thesis is substantiated, would confine the usefulness of this test within rather narrow limits.

¹ Falls: Jour. Infect. Dis., 1915, **16**, 466. Petersen and Short: Ibid., 1918, **22**, 147.

² Brit. Jour. Exper. Path., 1922, **3**, 224.

³ Jour. Lab. and Clin. Med., 1922, **7**, 10; Jour. Dent. Res., 1922, **4**, 435.

⁴ Rose: Physiol. Rev., 1923, **3**, 544.

⁵ Arch. Dermat. and Syphil., 1923, **8**, 801.

CHANGES IN THE URINE.

The changes from the normal in the urine during infection reflect not only the renal condition but also diverse metabolic disturbances. The quantity voided in a given time is often diminished. Sometimes during convalescence from acute febrile diseases polyuria occurs. An intensely acid reaction may be observed in fevers and acute articular rheumatism. In pneumonia the chlorid content is decreased; sometimes the test for chlorid becomes negative. A similar though less marked decrease occurs in many acute febrile diseases, *e. g.*, scarlatina, measles, smallpox, typhus, typhoid, recurrent and malarial fevers. The elimination of phosphates is also diminished in most febrile diseases. Usually the decrease is proportional to the severity of the disease. The sulphate content of the urine may be increased as *e. g.*, in pneumonia and acute myelitis. Likewise the elimination of urea is increased in febrile diseases, notably so in the case of pneumonia. In acute febrile diseases, such as pneumonia and typhoid fever, a large increase of creatin and creatinin output may occur; a decrease is observed in phthisis. Albuminuria may occur in almost any of the febrile infections. This phenomenon is very generally observable. Simon¹ from whom most of this paragraph is summarized, describes a digestive glycosuria in numerous febrile diseases, *e. g.*, pneumonia, typhoid fever, acute articular rheumatism, scarlatina and tonsillitis. The amount of glucose found usually runs from 0.5 to 3 per cent; one case is on record in which 8 per cent was present. A glycosuria also occurs in cerebral and spinal meningitis, apparently attributable to irritation of nervous centers. Possibly a similar type is seen in typhoid, scarlatina, measles, cholera, diphtheria, influenza, and especially malaria; in which cases the glycosuria may represent disturbance of nerve centers by toxic products.

Indican in the urine in amounts above the normal limit is usually regarded as an index of intestinal putrefaction. Its ordinary source is the indol formed by intestinal bacteria from protein foods. In clinically significant quantities, indicanuria may suggest chronic intestinal stagnation, a condition in its sequels not without similarities to focal infection elsewhere in the body.

Acetonuria is common in the febrile diseases: Typhoid, pneumonia, scarlatina, measles, acute miliary tuberculosis, acute articular rheumatism, and septicemia.

The "diazo" reaction in the urine may be indicative of an abnormal

¹ Clinical Diagnosis, 9th ed., Lea & Febiger, Philadelphia, 1918.

katabolism of body proteins. It is positive in about 97 per cent of cases of typhoid fever and may also occur in measles, scarlatina, pneumonia, and erysipelas.

Microscopical examination of urinary sediments is useful in revealing pathological changes during infection. Numerous pus cells, of course, indicate pyogenic infection of the genito-urinary tract. "Casts" are demonstrable in a great variety of abnormal conditions and carry often considerable clinical significance.

The Urine in Oral Disease.—It must not be overlooked that the condition responsible for the change in the urine may also act as a predisposing factor for oral disease. This is particularly true in the case of diabetes mellitus, in which a glycosuria exists, and in various renal conditions exhibiting an albuminuria, perhaps grouped together by the term "Bright's disease." In these instances oral infections occur, in part attributable to the systemic condition. Urinary changes have also been described as the "result" of oral infections.

Medalia¹ in 9 of 14 incipient cases of pyorrhea alveolaris found an increased amount of indican. In none of these cases was there any albumin or sugar. The reaction was neutral in 3 and acid in 11 cases. Of 13 moderately advanced cases the indican was abnormally high in 4. The urinalysis of 53 advanced cases gave the following results: 44 acid, 7 alkaline, and 2 neutral; 27 showed marked indicanuria; 3 showed albumin and 2 showed sugar.

Of 30 cases of diabetes, studied by Roddy, Funk and Kramer² all but 3 had more or less conspicuous evidences of pyorrhea alveolaris.

Daland³ observed that a "common systemic result of a focal infection is an acute catarrhal nephritis, the urine usually containing erythrocytes, casts, and albumin."

Dick and Dick⁴ found considerable numbers of bacteria in the urine in 66 per cent of patients with evident foci of infection. This observation is interpreted as indicating that in diseases where migration of bacteria from foci of infection takes place, the bacteria are often present in the urine. Goadby has expressed almost exactly the same view.⁵

Sixty-two per cent of a series of 200 clear-cut cases of chronic oral infection were found by Crance⁶ to show a definite albuminuria. Glycosuria was demonstrated in only 2 cases (1 per cent).

¹ Dent. Cosmos, 1913, **55**, 24.

² New York Med. Jour., 1916, **104**, 433.

³ Canada Lancet, August, 1917.

⁴ Arch. Int. Med., 1917, **19**, 493.

⁵ Diseases of the Gums and Oral Mucous Membrane, Oxford Med. Pub., 1923.

⁶ Med. Rec., October 8, 1921.

LOSS OF WEIGHT.

This change is a frequent accompaniment of the severer types of acute infections and of the chronic infections of longer duration. It is determined by a multiplicity of factors, *e. g.*, the increased rate of metabolism associated with many febrile conditions, diminished ingestion from anorexia, angina, trismus, etc., disturbed digestion and assimilation, the vomiting and diarrheas of gastro-intestinal infections, the dehydration of the tissues in dysenteries and in cholera. It not infrequently happens that the first symptom which directs the patient to the physician in early pulmonary tuberculosis is a rather rapid loss of weight. In milder chronic infections this tendency may express itself not so much as a conspicuous loss of weight as an inability to regain a normal weight level.

HISTOLOGICAL AND CYTOLOGICAL CHANGES.

These comprise much of the subject matter of formal, classical pathological anatomy, and consequently they will be most cursorily treated here. It is serviceable to divide them into retrogressive and progressive. The retrogressive changes represent damage to the host induced by the infection; while the progressive changes, including inflammation, repair, and regeneration, are usually interpreted as representing defensive, protective, and reparative reactions on the part of the host.

Cloudy swelling, the commonest of the retrogressive changes, is frequently associated with the acute febrile infections, and at times seems to go directly over into fatty degeneration. Amyloid degeneration is characteristic in long-standing, chronic infections, *e. g.*, tuberculosis, actinomycosis and pyogenic osteomyelitis. The necrosis within the miliary tubercle and the focal necroses in the mesenteric lymph nodes, spleen and liver occurring during typhoid fever, are among the commonest effects of these infections.

The subjects of inflammation, repair and regeneration are intimately associated. We shall devote a little attention to inflammation because it represents what is probably an invariable, initial, local response to the presence of bacteria.

INFLAMMATION.

The most frequent, local response of living tissues to infection is inflammation. This is the commonest of all pathological processes

and infection is the commonest, natural cause of inflammation. As a consequence, adequate descriptions are available in any of the standard texts on general pathology. The view is almost universally held that it is essentially an adaptive process directed toward destroying or removing or delimiting the influence of the irritant (usually bacteria and their products) and toward the repair of damage done. The fluid part of the inflammatory exudate acts as a diluent for the irritant and also serves mechanically to flush out the irritant onto a free surface or into lymphatic channels. The fluid also, by virtue of its alexin—and specific antibody content: Antitoxins, agglutinins, precipitins, amboceptors, opsonins, etc., facilitates the destruction of the invading microorganisms or of the products of their activity. Various enzymes may be present at times in this exudate, aiding in the removal of necrotic masses. Finally the fibrinogen content of some types of inflammatory exudates plays a part in walling-off the irritant and in furnishing a scaffolding for the beginning of reparative processes.

The functions of only some of the cells of these exudates are known, but they are highly important. These elements are almost without exception derived by escape from the bloodvessels. Metchnikoff¹ regarded the inflammatory process as essentially a mechanism for the mobilization of phagocytic cells at the site of the irritant. Krogh² speaks of the emigration of leukocytes as “one of the central, if not *the* central reaction in inflammation.” The polymorphonuclear neutrophils and the endothelial leukocytes are conspicuously phagocytic, ingesting the invading bacteria. This behavior will be considered at length in a separate section on phagocytosis. The functions of the other types of cells is less obvious. The lymphocyte, more abundant in chronic lesions, is not phagocytic. It is suggested³ that it acts as an affixer of toxin. The role of the plasma cell, a type not occurring in the circulating blood, but probably related to the lymphocyte, is problematical and wholly obscure. The same characterization must be made of the eosinophilic cell, except that it appears to share in the defense against various animal parasites.

An exudate rich in polymorphonuclear neutrophils is characteristic of the *purulent* or *suppurative* type of inflammation. This is commonly caused by the so-called pyogenic bacteria, *e. g.*, *Staphylococcus aureus*, *albus* and *citreus*, *Streptococcus pyogenes*, *pneumo-*

¹ Leçons sur la pathologie comparée de l'inflammation, Masson, Paris, 1892.

² The Anatomy and Physiology of Capillaries, Yale Univ. Press, 1922.

³ Bunting: *Physiol. Rev.*, 1922, **2**, 505.

coccus, *Micrococcus tetragenus*, *M. catarrhalis*, gonococcus, meningococcus, *Pseudomonas pyocyanea*, and *Bacillus pestis*. This purulent reaction is very common and is probably the best known of all the inflammatory types. The exudate itself is ordinarily referred to as pus; a liquid in which are suspended many cells and cell-fragments. The liquid part differs from blood serum chiefly in the substances added to it through proteolytic changes, and also in that it has lost its antiproteolytic property, containing instead free leukoprotease. Pus serum shows an increased proportion of fatty matter and extractive substances. All the numerous enzymes of the blood plasma, the leukocytes and the tissue cells are present in

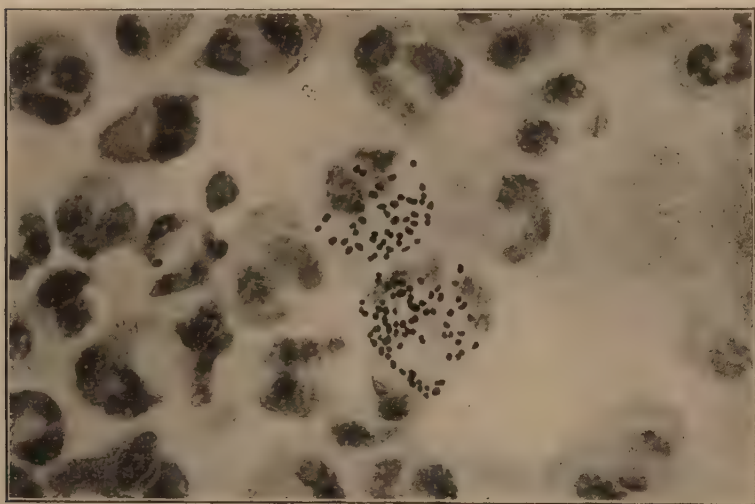


FIG. 40.—Purulent exudate. (Kendall.)

pus. The cells found in pus are living, normal or in various stages of degeneration, and dead, intact or fragmented. Bacteria, tissue cells and any or all of the various types seen in inflammation, *but with the polymorphonuclear neutrophils by far predominating*, comprise the cellular elements. Suppuration is the result of three processes: (1) The necrosis of cells; (2) the local accumulation of leukocytes and a modified blood plasma, and (3) the digestion of the necrotic cells, fibrin and tissue elements by enzymes. These enzymes are derived from three sources: (1) The leukocytes; (2) the infecting bacteria (if such be present), and (3) the fixed tissue cells.¹ Pus has such a characteristic appearance that usually

¹ Wells: Chemical Pathology, Philadelphia, 4th ed., 1920, p. 268, *et seq.*

simple, naked-eye inspection suffices for its recognition. In case there be any question, as when the quantity is small or blood-stained, simple microscopic examination of a smear, using at least a 4 mm. objective is the only way to reach a reliable decision. This recourse to the microscope is often necessary in oral infections, particularly when the dentist is desirous of checking up on the efficacy of his treatment.

The protective phase of the inflammatory reaction may be seen in the diminished rate of absorption from the involved area. Park, Famulener, and Banzhaf¹ have shown this in the case of concentrated diphtheria antitoxin (globulin). Opie² observed that when a foreign protein (horse serum or crystalline egg albumen) is injected into the skin of an immune animal, an acute inflammation (Arthus phenomenon) occurs at the site of injection. The antigens (*irritants*) are fixed at the site of entry and destroyed. They cannot be recovered from the blood of the general circulation. On the contrary when these same substances are injected into a normal (non-immunized) animal no inflammation results and they are demonstrable in the blood stream. As far back as 1897, Cobbett and Melsome³ obtained evidence indicating that even a non-specific inflammation, such as that produced by mustard oil, would prevent experimental infection. Chesney (see *infra*) likewise has made observations which suggest that inflamed areas as in a healing wound, are not particularly favorable sites for the growth of the ordinary microorganisms contaminating the skin. On the other hand, however, there is evidence that in some instances the seat of an inflammatory reaction constitutes a favorable site for the implantation of an infection. Teague and Goodpasture⁴ have noted this in the case of the virus of herpes zoster, and Chesney and Kemp⁵ have found that old granulating wounds in rabbits offer a particularly suitable terrain for syphilis inoculation. The chancre develops relatively soon and attains a greater size than the lesion occurring on the basis of a fresh wound.

The reparative phase of inflammation is dependent upon the proliferative activities of the affected cells. The fibroblast is particularly sensitive to stimuli of this nature and readily undergoes mitosis. The resulting white fibrous connective tissue may more or less successfully wall off the irritant or accomplish a more or less satisfactory restitution of the damaged part. The encapsulation of abscesses, of tubercular foci, and of infected thrombi and infarcts,

¹ Jour. Infect. Dis., vol. 14, 338.

² Jour. Exper. Med., 1924, 39, 659; Jour. Immunol., July, 1924, vol. 9.

³ Jour. Path. and Bacteriol., 1896, 3, 39.

⁴ Jour. Med. Res., 1923-1924, 44, 185.

⁵ Jour. Exper. Med., 1925, 41, 487.

the changes in the heart valves leading to insufficiency or stenosis, the formation of thoracic or peritoneal adhesions, the obliteration of the lumen in chronic appendicitis, are all instances of this proliferative power of the fibroblast, released under the conditions existing in inflammation, particularly when this reaction runs a chronic course. The fibroblasts participating are probably largely those resident at the affected site, preceding the arrival of the irritant; but wandering mesenchymal cells or even endothelial cells or endothelial leukocytes may under these conditions become transformed into fibroblasts, proliferate and share in the attempt at repair. Carrell¹ has found that leukocyte extracts or peritoneal exudate or connective tissue invaded by leukocytes, possess the power of increasing the rate of multiplication of fibroblasts *in vitro*.

Cell-multiplication need not be limited to the fibroblast. The vascular endothelium possesses high potentialities for proliferation. The formation of new bloodvessels or of capillary networks is one of the commonest manifestations of a subacute or chronic inflammation. The complex of new-formed capillary loops supported on a fibroblastic framework, in whose meshes lies an inflammatory exudate exhibiting the various types of cells enumerated above, constitutes what has long been known as granulation tissue. The proliferation of endothelial cells in typhoid fever, according to Mallory² accounts for the characteristic intestinal ulcers and the focal necroses seen in the mesenteric lymph nodes and liver in this disease. The mitosis and multiplication of epithelial cells is frequently encountered in inflammation. This is well seen, for instance, in the skin and mucous membranes in cases of lupus or other types of tuberculosis. In cases of inflammation involving glands proliferation occurs in the epithelium lining the ducts. This is well seen in the liver, in which the bile ducts may form long, tortuous channels or cords.

THE INFLAMMATORY PHENOMENA IN ORAL TISSUES.

There is nothing peculiar in the inflammations which affect the gingivæ. The epithelialization of pyorrhetic pockets may be noted in passing as evidence of the proliferative phase. The "purpose" of this new epithelial layer is to protect the underlying connective tissues, but inasmuch as it prevents close approximation or fusion of the gingivæ to the surface of the denuded root, it interferes with the realization of a legitimate goal of dental treatment. The same condition is observable in fistulous tracts, which likewise

¹ Jour. Exper. Med., 1922, **36**, 385.

² Ibid., 1898, **3**, 611.

require deëpithelialization in treatment. The tissue reaction of periapical tissues to infection cannot be disposed of so easily. The histological picture described above as granulation tissue is applicable to most instances of periapical inflammation, which run a sub-acute or chronic course. Fibroblastic and more or less vascular proliferation are the most prominent features. In the meshes of



FIG. 41.—Granulation tissue of periapical infection. *a*, fibroblast; *b*, plasma cell; *c*, hyalin bodies; *d*, eosinophils; *e*, lymphocytes; *f*, leukocytes between epithelial cells; *h*, epithelial cells. (Thoma.)

this new growth, are to be found the various types of inflammatory cells. Plasma cells usually are very numerous. Here and there, in sections, one sees rather discrete groups of polymorphonuclear cells; a condition indicative of an acute exacerbation superimposed upon a more chronic process. The most peculiar feature, however, of periapical inflammation is the frequent presence of masses and

strands of epithelial cells. These are ascribed to the proliferation which has been induced in the epithelial cell-nests of the alveolodental periosteum, the normally quiescent remnants of Hertwig's epithelial sheath. The growth of these epithelial masses with consequent liquefaction of their contents and the assumption of a secretory function, result in the formation of the common dental cyst. Henrici and Hartzell¹ offer the suggestion that these epithelial structures represent an attempt to protect the deeper tissues of the jaw from infection sequent to the exposure and death of the pulp. The epithelial proliferation may succeed in interposing a layer of stratified squamous epithelium (highly impermeable to bacteria) between the exposed connective tissues and the outside world.

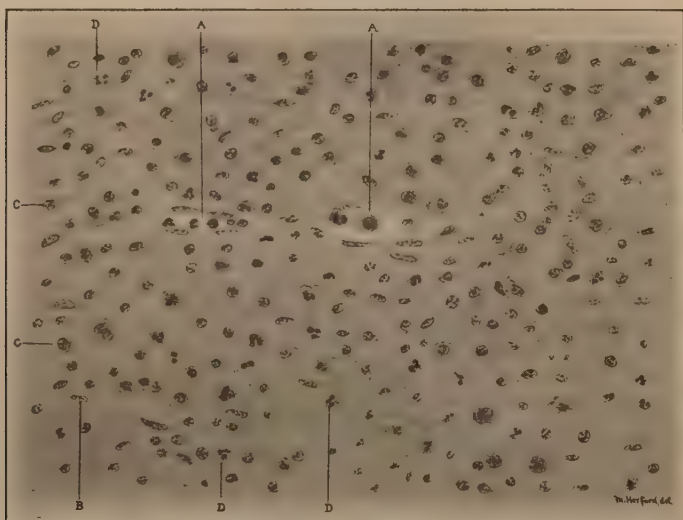


FIG. 42.—Granulation tissue of periapical infection. *a*, vessels containing lymphocytes and polymorphonuclears; *b*, fibroblasts; *c*, plasma cells; *d*, polymorphonuclears. (Thoma.)

The periphery of this periapical new growth of granulation tissue is usually sharply demarcated by dense, concentric layers of white fibrous connective tissue, not infrequently showing some hyalinization. This is an instance of the attempt of an inflammatory reaction to wall-off an irritant, the clinical significance of which fact taken in conjunction with the reaction in its entirety, has been emphasized by Price. According to this author, a roentgenographically conspicuous reaction is less portentous than a periapical infection with little or no reaction.

¹ The Microscopical Anatomy of Chronic Periodontitis and the Pathogenesis of Dental Root Cysts, Jour. Nat. Dent. Assn., 1917, 4, 1061.

CHAPTER IX.

FACTORS PREDISPOSING TO INFECTION.

It is a popular, naïve belief that a particular event or object, B, is the result of a particular cause, A. The study of the principles of science has shown that such a belief is not strictly or literally justified. Put positively, a fundamental idea of the philosophy of science is that each event or object, B, is the resultant of the combined action of a multiplicity of factors, $A_1, A_2, A_3, \dots A_n$. This view-point has long been in effect in the consideration of the causes of disease and is expressed in the recognition of *predisposing* as well as of *exciting* factors. The meaning of this and the distinction will become apparent from a concrete illustration. In the case of tuberculosis, the exciting cause is the tubercle bacillus. The presence of this microorganism is an indispensable condition for the development of this infection. All the predisposing factors may be operative but without the active collaboration of the tubercle bacillus there can be no tuberculosis. Poverty in this case is a potent predisposing factor. In this sense poverty implies poor food, crowding, poor ventilation, dirty clothing, overwork, ignorance of the principles of personal hygiene, and inadequate protection from the weather. These and still other conditions or circumstances are factors favoring or predisposing to tubercular infection. This illustration is given to aid in understanding the significance of the various items listed below.

SOME FACTORS PREDISPOSING TO INFECTION.

1. **Species, in the Taxonomic Sense.**—Different species of animals and plants suffer from different infections. Tuberculosis is common to the human being, other primates and some herbivora. The guinea-pig is very susceptible while another rodent, the rat, is highly resistant. The carnivora are in general highly immune.

2. **Race.**—The several races within a species at times are not equally susceptible to the same infection. The Negroes and Eskimos are very susceptible to pulmonary disease. The natives of the tropics exhibit a high resistance to the infections peculiar to the tropics while by way of contrast the Caucasians relatively readily contract such diseases. Such differential resistances may be due

to other factors than hereditary, biological characteristics. A classical instance of racial difference is afforded among the sheep. The European races of sheep are highly susceptible to anthrax infection while the Algerian sheep are very resistant.

There seem to exist racial differences which considerably influence the development of natural immunity, as indicated by the Schick test, to diphtheria. Negro children, even when living in the crowded conditions of congested neighborhoods, show a high proportion of susceptibles. Children of Italian extraction, living in the crowded East Harlem section of New York City, gave the lowest percentage of susceptibles. About one-third of the children of Bohemian or Irish extraction were susceptible. Attention was called to these differences by Zingher¹ and confirmatory experiences have been reported by Sears² and by Ceconi at Boston, Mass.

3. **Age.**—Most of the deaths of infants less than one year of age are due to gastro-intestinal infections. These are more common at this time of life than at any other age. Ficker³ and Hilgermann⁴ showed that bacteria can more easily penetrate the intestinal mucosa of young than older animals. In early childhood, puberty, and early adult life the individual is more susceptible to tuberculosis than a more mature adult. Typhoid fever is more common to the fully matured than to younger people. Pneumonia makes its greatest ravages among the very young and the very old. Appendicitis shows variations according to age, being more frequent at about the age of eighteen and declining in incidence from then on. In childhood we have the so-called children's diseases: Measles, chickenpox, whooping-cough, mumps, diphtheria, and scarlet fever. Age as a predisposing factor may act in two ways. In a strict biological sense, age would only predispose to infection when it did so by virtue of certain physiological peculiarities of the particular age under consideration. The term is, however, often used to cover such predispositions not biologically inherent, but as occur indirectly because of social customs. For instance our "children's diseases" may predominate in childhood because assembling and confinement in the school room affords greater opportunity for exposure. It must not be forgotten in this connection that one reason why "children's diseases" are rarer with increasing age, is that most individuals acquire them early and because of the lasting immunity are forever after less susceptible.

¹ Jour. Am. Med. Assn., 1921, **77**, 835.

² Am. Jour. Pub. Health, 1924, **14**, 210.

³ Arch. f. Hyg., 1905, **52**, 179.

Ibid., vol. **54**, 335.

The influences of age and sex upon disease is indicated by the following table.¹ It is impossible to tell what fraction of the percentages given in the first and last columns is due to infection, but it is known that an infectious factor is important in most deaths.

Males.		Age group, years.	Females.	
Per cent of all biologically classifiable deaths due to the break-down of the specified organ system.	Organ system concerned in largest proportion of fatalities.		Organ system concerned in largest proportion of fatalities.	Per cent of all biologically classifiable deaths due to the break-down of the specified organ system.
68.8	Alimentary tract	0 to 1	Alimentary tract	40.6
50.1	Respiratory	1 to 4	Respiratory	51.3
41.2	Respiratory	5 to 9	Respiratory	42.5
27.1	Respiratory	10 to 14	Respiratory	33.3
43.6	Respiratory	15 to 19	Respiratory	43.8
52.6	Respiratory	20 to 24	Respiratory	46.0
49.7	Respiratory	25 to 29	Respiratory	44.2
45.6	Respiratory	30 to 34	Respiratory	39.5
39.9	Respiratory	35 to 39	Respiratory	33.2
33.3	Respiratory	40 to 44	Respiratory	27.5
28.0	Respiratory	45 to 49	Respiratory	22.1
23.6	Respiratory	50 to 54	Alimentary tract	21.6
25.0	Circulatory	55 to 59	Alimentary tract	22.6
28.4	Circulatory	60 to 64	Circulatory	24.4
30.9	Circulatory	65 to 69	Circulatory	25.6
32.5	Circulatory	70 to 74	Circulatory	28.0
32.9	Circulatory	75 to 79	Circulatory	28.4
33.0	Circulatory	80 to 84	Circulatory	30.4
		85 to 89	Circulatory	30.8

4. **Sex.**—Of course different infections are associated with the distinctive structures and functions of each sex. For instance: Puerperal septicemia or a salpingitis can occur only in the female. Similarly at times rapidly progressing, fatal cases of tuberculosis make their appearance either during or shortly after the termination of pregnancy. Pregnancy has often been associated with an increased susceptibility to dental caries, a gingivitis and a stomatitis. By way of contrast, sycosis vulgaris is limited to males, with the possible exception of the bearded lady. Aside from this type of sex predisposition there are other types whose nature is less readily understood. Appendicitis is appreciably more frequent in the male than in the female. A curious, related phenomenon is that about 82 per cent of all typhoid carriers are females. Guenther² has collected evidence indicating that diphtheria is more frequent in females during the first half year of life. The *morbidity* in females

¹ Pearl: *Biology of Death*, 1922, p. 136 (Table 11).

² *Zentralbl. f. inn. Med.*, 1924, 45, 290.

progressively increases from the fifth year onward. In spite of this the *mortality* in boys is higher because of the greater frequency of laryngeal involvement. What are apparently sex differences in respect to relative susceptibility or resistance to a given infection may at times be more properly regarded as occupational differences. Pulmonary tuberculosis in general is much more frequent in the male. An adequate explanation of this may be that in modern civilized society the tubercular hazard associated with occupation is the greater for the male.

5. **Fatigue.**—Fatigue is a predisposing factor to infection. This has long been recognized clinically. As an instance of its experimental demonstration, we may cite the work of Abbott and Gildersleeve.¹ Muscular fatigue was induced in rabbits by keeping them for varying lengths of times in a motor-driven, revolving drum. They found that prolonged muscular exercise *followed* by the injection of pyogenic bacteria may favor pyogenic infections, but that violent muscular exercise *following* inoculation is more apt to result seriously. It was observed that the opsonizing power of the blood may be markedly diminished by severe muscular exercise.

6. **Infection.**—In some cases, as probably in pneumonia, recovery may leave the individual more susceptible, than previously, to reinfection with the same microorganism. One infection also frequently predisposes the patient to infections with other microorganisms. This phase of the problem is difficult to separate from the synergistic phenomena of mixed infections. Influenza, particularly as illustrated in the great pandemic of 1918, rendered the patient susceptible to infection with hemolytic streptococci, pneumococci and Friedländer's bacillus. The high mortality was largely due to these secondary, pulmonary involvements. In a similar fashion typhoid fever and measles predispose to pneumonia and tuberculosis. Tetanus bacilli introduced into an animal in pure culture may fail to give rise to tetanus, which, however, may be routinely induced if pyogenic staphylococci accompany the tetanus bacilli. Roux and Vaillard² found that guinea-pigs which had been debilitated by anticholeric vaccination, may succumb to injections of toxin-antitoxin mixtures, inoffensive for the normal guinea-pig. Muir³ reports a case showing the influence of one infection upon another. A female patient had a small, anesthetic, leprosy patch on her arm, which was the only sign of the disease. Typhoid fever

¹ Univ. Pennsylvania Med. Bull., 1910, 23, 169.

² Cited by Bordet, *Traité de l'Immunité*, Masson et Cie, Paris. 1920, p. 530.

³ *Lancet*, 1925, 208, 169.

developed and during the afebrile convalescence, an erythematous rash spread in patches all over the body within a few weeks. The patches were raised and contained lepra bacilli. As the patient recovered her strength the patches faded and became flattened to the level of the surrounding skin. The typhoid infection had lowered her resistance and made her tissues a suitable soil for the growth of *B. lepræ*. As her resistance returned the soil became unsuitable once more and the lepra bacilli began to disappear.

7. Diabetes Mellitus.—This disturbance of carbohydrate metabolism has long been known to make the individual more susceptible to infection with the pyogenic cocci. Rosenau¹ has pointed out that deaths from diabetes show a curve corresponding to that of the incidence of pneumonia and bronchitis. The inference is that the ultimate cause of death was an infection implanted upon the debilitated diabetic. Surgeons are hesitant about operating on diabetics. Pyorrhea alveolaris is highly refractory in diabetics to all local treatment. Although oral care should not be neglected, energetic treatment is often unavailing until the metabolic disturbance is controlled by a proper dietary regimen and insulin administration. In all pyorrhetic cases which prove extraordinarily obstinate, an examination for glycosuria is indicated.

A naïve suggestion to account for the heightened susceptibility among diabetics is that the increased sugar content of the blood enhances the nutrient value of this fluid for the bacteria. This view, supplemented by the hypothesis that the increased viscosity of the blood in diabetics favors sluggish capillary circulation with consequent relative stasis, has been advanced by Rubin and Arkin.² These authors report that glucose in the blood even in high concentrations, has no influence on the phagocytic function of the leukocytes. This latter finding is only confirmatory of Handmann's³ observation that the addition of sugar to blood *in vitro* did not diminish its bactericidal or opsonic properties.

In the combined light of the data available it is probable that the increased susceptibility to pyogenic infection in diabetics is due not to the increase in blood-glucose but rather to some deleterious effect exerted by the disease upon the formation of protective substances, bactericidal and opsonic, in the body. In support of this view may be cited the work of Sweet⁴ who found that while the bactericidal properties of the blood were not changed by the

¹ Scient. Monthly, 1925, **20**, 201.

² New York Med. Jour. and Med. Rec., August 15, 1923, p. 212.

³ Deutsch. Arch. f. klin. Med., 1911, **102**, 1.

⁴ Jour. Med. Res., 1903, **10**, 255.

hyperglycemia induced by epinephrin injections they are definitely diminished after excision of the pancreas (the organ involved in diabetes mellitus). Da Costa and Beardsley¹ in natural human cases found a subnormal opsonic index, which was particularly low in the more severe cases studied.

That the increased susceptibility in diabetics may not be limited to pyogenic bacteria is indicated by the work of Takemura.² Bovine tubercle bacilli were introduced intravenously and intrathoracically into rabbits in which diabetes had been experimentally induced. The diabetic animals contracted tuberculosis more easily than the controls. There was marked depression of the phagocytic power of the leukocytes in the serous fluid of the thoracic and abdominal cavities of the diabetic rabbits. Takemura also observed that sugar (glucose) in the culture medium neither hindered nor accelerated the growth of the tubercle bacillus.

8. **Heredity.**—Probably all genuine cases of specific or racial susceptibilities or immunities are of a hereditary character. The most important conception bearing on the relation of heredity to infection is that no infectious disease can be inherited in the strict, biological sense of the word heredity. Whether or no we say an infection is inheritable, is, of course after all, a matter of the definition of "inheritable." We can "inherit" an infection in a property sense, as we can legally in a "capitalistic" nation inherit real estate; but we cannot inherit an infection in the sense that we biologically inherit color blindness, or our "blood type" or polydactylism or any other of many physical peculiarities. Inasmuch as the subject matter of medicine belongs to the field of biology rather than to the field of statutory legislation, we should in the present case accept the biological, rather than the legal, conception of inheritance. For an infectious disease to be biologically inherited, it would be necessary for one living organism (the parasite) to become an integral part of the germ-plasm of another living organism (the host). This is biologically inconceivable, therefore no infectious disease can be inherited.

It is worth while to spend some time on this topic because two such socially important diseases as syphilis and tuberculosis are commonly and widely described as inheritable. It is an unfortunate fact that offspring of syphilitic parents may at birth or shortly thereafter exhibit the stigmata of syphilis. The possibility of postnatal infection may be eliminated. In these cases we are dealing with infection antenatally acquired, *e. g.*, by placental penetration

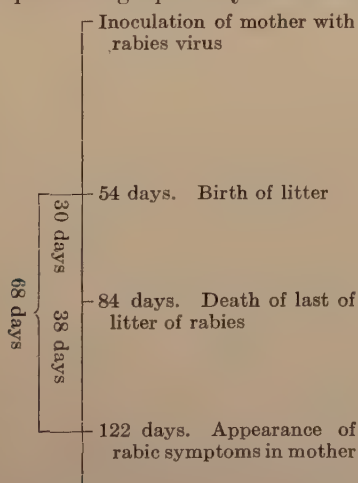
¹ Am. Jour. Med. Sci., 1908, **136**, 361.

² Japan Med. World, September 7, 1919,

of *Treponema pallidum* from the maternal circulation into the fetal. The term hereditary or heredo-syphilis should be discarded. In its place, the term congenital syphilis is probably the best available, although as it is non-committal and properly includes genuine hereditary conditions it is not ideal.

Syphilis is not the only instance of an antenatally acquired (not inherited) infection. The classical example is afforded by pébrine, a disease of silk-worms, controlled by Pasteur's efforts. In this case we are dealing with a protozoan, not a bacterial infection. The microorganism in question, *Nosema bombycis*, invades not only the digestive tract of the insect, but also other parts of the body including the ovaries. "Spores formed in the ovarian eggs germinate in the larvæ that hatch from the eggs and in this way pass from one generation to the next."

Spirochetes in recurrent fever of man can penetrate the placenta, and the same mechanism has been described by Konradi (1916) for the transmission of rabies to the fetus. Remlinger¹ has supplied some experimental evidence. Animals inoculated with rabies may not display any signs of the disease for from one to three months, while their young born in the interim present rabies and may have died before their mother shows any signs of the disease. A guinea-pig was inoculated with rabies. Fifty-four days later it cast a litter; the last of this litter was dead of rabies, eighty-four days after the inoculation. Rabic symptoms did not appear in the mother until one hundred and twenty-two days after the inoculation, *i. e.*, thirty-eight days after the death of the last of the litter. The following scheme represents graphically the above facts.



¹ Bull. de l'Acad. de méd., Paris, 1919, 81, 437.

Whitman and Greene¹ reported a case of disseminated miliary tuberculosis in a still-born fetus. The histological changes establish its transplacental origin. The passage from the mother through the placenta to the fetus accounts for the case reported by Block.² The mother had pulmonary phthisis; the infection probably first made itself known in the offspring eight days after birth. The localization was abdominal.

It is possible for the fetus to become infected with the virus of smallpox while the mother is in the incubation stage of that disease. Four such cases are cited by Cappellani.³ In 1 of these cases, the fever appeared in the child twenty-four hours after birth and typical smallpox developed although the mother failed at any time to present the clinical signs of this infection.

Among the other infections which are known to be occasionally acquired *in utero*, are scarlet fever, erysipelas, acute rheumatism, cholera, typhoid fever, influenza, cerebrospinal meningitis, mumps, and yellow fever. Even prenatal infestation with parasitic worms has been described.⁴

These cases are not regarded as inherited infections. They are admittedly antenatally *acquired* infections and yet they differ in no essential respect from what is generally termed "heredo-syphilis." They are introduced here to emphasize the incorrectness of this latter term and to illustrate the fact that the possibility of antenatally acquiring an infection is by no means limited to syphilis.

In this connection it should be noted that specific antibodies which raise the resistance against one or another of the infections are transmissible from the immune mother to the fetus through the placenta or to the new-born in the milk. These are not to be interpreted as instances of inheritance of an immunity but rather as instances of *passive* immunization (see *infra*).

Next to syphilis, tuberculosis is the infection to which has most frequently been assigned a hereditary origin. The reason for this in the days before the biological concepts of heredity had been clearly defined, is easy to understand. The incidence of tuberculosis is strikingly familial. The children of parents, one or both tubercular, exhibit a far higher rate of phthisis than do the children of the non-tubercular or even of the general population. As a striking instance, Menetrier and Bertrand-Fontaine⁵ contribute an instance where

¹ Arch. Int. Med., 1922, **29**, 261.

² Ztschr. f. Kinderhkl., 1924, **37**, 242.

³ Pediatria, Naples, 1919, **27**, 193.

⁴ Cort: Jour. Am. Med. Assn., 1921, **76**, 170.

⁵ Bull. de l'Acad. de méd., 1924, **91**, 275.

6 pairs of male twins were born of a tuberculous father. Nine of them died from *pulmonary* affections. The other 3 sons were killed in the war, else they might have similarly succumbed. Apparently supporting the contention of hereditary transmission is the discovery by Karl Pearson¹ that the statistical correlation between parent and offspring in the matter of tuberculosis is of the same intensity as the hereditary correlation between admittedly inherited characters in man. Furthermore, the correlation between husband and wife in the matter of tuberculosis is far less intense than that between parent and offspring. Pearson's findings probably correspond very closely to reality, and yet they in no way force us to accept the view that tuberculosis is inherited biologically. Their just interpretation corroborates the opposed view, viz; that infections are not inherited.

The higher incidence of tuberculosis among the offspring of tubercular parents than among the offspring of the general population is adequately accounted for by two phenomena: (1) Certain morphological or physiological characteristics are inheritable, which characteristics render their possessor more susceptible to the tubercle bacillus, and (2) the children of tuberculous parents are more constantly and intensely exposed to tubercular infection than are the children of non-tubercular families. It might, however, be urged that the role of exposure could be discounted. By reference to Pearson's data it is seen that although the exposure of one parent, when the other was tuberculous, was at least as constant and intense as that to which the children were subjected, nevertheless tubercular infection was relatively far less frequently contracted by the originally non-tubercular member of the marital couple than by the offspring of that union. The facts set forth in the above sentence certainly are compatible with the view of a genuine inheritance. But absence of incompatibility is not proof of correctness. The facts may be accepted essentially as presented, but the higher incidence among the offspring is due in part: (1) To the inheritance of a *predisposition* mentioned above and (2) probably in greater part to the greater susceptibility to tuberculosis among children than among adults.

The above considerations reflect the great complexity which is presented whenever we try to recognize and evaluate all the "causes" that are concerned in the consummation of an infection. It is easy in the case of open tuberculosis to understand the significance of increased exposure. Other things being equal, the greater the

¹ Lancet, 1920, 199, 891.

concentration of tubercle bacilli in one's environment the greater will be the likelihood of successful invasion. The meaning of the inheritance of a predisposition and the nature of that predisposition are less obvious.

A tubercular diathesis or a lessened resistance to tubercular infection has long been discussed. Many attempts have been made to correlate the shape of the thorax or the quality and pigmentation (among Caucasians) of the skin and hair with this susceptibility. In any large group of autopsies on individuals with phthisis, one is struck with the delicacy and elasticity of the aorta and the relative smallness of the heart. Surmises have been advanced as to the effect of this upon the pulmonary circulation. In general we may say that our knowledge of the nature of this diathesis is less satisfactory than is our evidence of its existence. Hauser¹ on the basis of a rather extended study concluded that a tendency or predisposition to contract tuberculosis might be inherited. Govaerts studied 214 families, in which 185 tuberculous matings and 29 non-tuberculous matings were found representing 5629 individuals. He became convinced of the reality of a tuberculous diathesis, which although not always inherited in the same degree, is nevertheless inherited in different degrees depending on the gametic constitution of the individual. A great deal of careful thought has been given to this problem by Pearl.² It is unnecessary for our present purpose to present his data or to summarize his argument. What is pertinent is the conclusion "that the inherited constitution of the individual is a factor in the problem of a great deal more than . . . negligible importance." The subject of the nature and characteristics, predisposing to tuberculosis, the tubercular diathesis or constitution, has been extensively reviewed by Carl Hart.³

Experimental evidence has been adduced by Wright and Lewis⁴ supporting the view that inheritable, constitutional or diathetic factors serve to predispose their possessor to tubercular infection. Using guinea-pigs which are highly susceptible to this infection, these authors by selective inbreeding obtained families or strains which exhibited marked differences in resistance, which differences were transmitted to offspring in a manner suggestive of a Mendelian dominant.

As another instance of experimental confirmation of the general

¹ Deutsch. Arch. f. klin. Med., 1898, **61**, 221.

² Chapter X in Studies in Human Biology (Baltimore, 1924, p. 273), based on an article appearing in the Am. Rev. Tuberc., 1920, **4**, 688.

³ Ergebn. d. allg. Path., 1910, **14**, 337.

⁴ Am. Naturalist, 1921, **55**, 20.

thesis that resistance or susceptibility to infection may be in part hereditary, may be cited the work of Webster.¹ It was found that if mice surviving an ordinarily lethal dose of mouse typhoid bacilli are inbred and this process continued for a number of generations, the resulting offspring become progressively more resistant to mouse typhoid infection than similar control mice not selectively inbred in this particular. There is also some evidence that the ability of rabbits and guinea-pigs to form agglutinins against the typhoid bacillus may be increased by selective breeding.²

Hereditary predisposing factors exist for other infections than tuberculosis. This case has been developed at some length because it has been more thoroughly studied. Price and others have attempted in their work on evaluating oral infectious foci to demonstrate a hereditary diathesis for streptococcal infections. Increased susceptibility to endocarditis often appears "to run in families." In the case of diphtheria Zingher³ noted a marked tendency for the children of the same family to be either all susceptible or all resistant. Hirszfeld, Hirszfeld, and Brokman have made a most interesting study in this connection.⁴ They examined 50 families with reference to susceptibility (Schick) to diphtheria, and the blood-group or type represented. Their most significant discovery was that if one parent were resistant to diphtheria and the other susceptible, then the children possessing the blood-group of the susceptible parent were also susceptible, while those children possessing the blood-group of the resistant parent were usually, though not invariably, also resistant. Blood-groups or types are definitely recognized to be biologically inheritable; *ergo*, an hereditary factor enters into the susceptibility or resistance to diphtheria. We shall see later that a peculiar condition of hypersensitivity is known as anaphylaxis, and that this condition has very real role in infection. A tendency or a predisposition to the development of the anaphylactic state has been shown by Cooke and Vander Veer to be probably inheritable. This would be looked for in bacterial asthmas.

The subject of the relation of heredity to infection may be summarized as follows:

1. Genuine biological inheritance of an infection does not occur.
2. A predisposition, diathesis, or increased susceptibility to an infection may be inherited.

¹ Jour. Exper. Med., 1924, **39**, 879.

² Guyer and Smith: Jour. Infect. Dis., 1923, **33**, 498. Learmuth: Jour. Hyg., 1923, **33**, 100.

³ Jour. Am. Med. Assn., 1921, **77**, 835.

⁴ Compt. rend. Soc. de biol., 1924, **90**, 1198.

3. Cases described as an inheritance of an actual infection, are to be interpreted in the light of the following considerations: (a) The infection was acquired antenatally or parturiently; (b) it is an instance of the inheritance of a predisposition to an infection, on which the actual infection has been implanted; (c) the exposure of children to infection in a household where one or both of the parents have an infection is naturally very great.

9. **Intoxications.**—Overindulgence in alcohol has for many years been regarded as a factor in lowering the resistance to infections. This condition does not require the advanced, morphological alterations seen in the heart, liver, and kidneys of the chronic inebriate. It has been found by Stillman¹ that in mice intoxicated with alcohol inspired pneumococci persist in the lungs for a longer period and a fatal septicemia more frequently follows than is the case in not intoxicated mice. Similar results were obtained when hemolytic streptococci or influenza bacilli were substituted for the pneumococci. Mercurial poisoning predisposes to fuso-spirillary infections of the mucous membranes, manifesting itself as the well-known mercurial stomatitis. The phosphorus necrosis, particularly as it affects the mandible is of classic interest to the dentist. In this condition we encounter a pyogenic osteomyelitis; a circumstance which might be forecast considering that in phosphorus poisoning the alexin- and opsonin-content of the blood is reduced.² The inhalation of irritating or toxic gases, including general anesthetics, have determined respiratory and particularly pulmonary infections. Possibly pregnancy as a factor predisposing to infection (*e. g.*, tubercular) is to be regarded as a type of intoxication. Influenza when extraordinarily virulent as in the pandemic of 1918, always has a very dark prognosis in the pregnant.

10. **Trauma.**—Trauma or mechanical injury predisposes to infection. Bacteria may in this way be directly introduced into the body by puncture, section, laceration or abrasion. Even though the instrument accomplishing the damage be sterile, infection may be introduced from the skin or clothing which always harbor microorganisms. In the Russian-Japanese war it was found that in general the greater the degree of personal cleanliness observed by the soldier the less seriously infected were his wounds. In the World War of 1914–1918, tetanus and gas gangrene terribly increased the hazards of the wounded. The extensive lacerations associated with shrapnel fire were particularly favorable soil for

¹ Jour. Exper. Med., 1924, **40**, 353.

² Zinsser: Infection and Resistance, 3d ed., 1923, p. 379.

the anaërobic types producing gas gangrene. Perforating wounds of the thorax, if the hemorrhage be not fatal, are often followed by pleuritis and pneumonia. Similar wounds of the abdomen may result in peritonitis, in which streptococci or colon bacilli predominate. The virus of rabies is almost invariably introduced by the bite of a rabid dog, and septic processes are sometimes initiated by any sort of bite, including that of non-poisonous snakes. The existence of an Eskimo tribe was threatened because the ceremonial knife which was used to sever the umbilical cord had become infected with tetanus bacilli. Analogous accidents have occurred as sequels to ceremonial circumcision. Concussions of the brain and fractures of the cranium predispose to infectious meningitis, and the bronchopneumonias of infancy are often rapid sequels to some particularly hard fall. The localization of an infectious arthritis has been determined by mechanical injury to a particular joint. The clinician has long known this and it has been confirmed experimentally.¹

Trauma may also serve to relight an infection which has not entirely cleared up or it may determine a new localization of infection already present in the body. Blows on the thorax may predispose to tuberculosis at times, occasioning in industrial accidents nice controversies under various compensation and liability laws. Injury to a joint may sometimes result in tuberculosis of that joint in individuals who are frankly tubercular or even in those who previously had shown no signs of that infection. The pathogenesis of this is a particular case of what has been said above relative to arthritis.

Chronic appendicitis may present an acute exacerbation following abdominal blows.

In some cases of syphilis the central nervous system may be the more seriously affected; in others the vascular system, appearing as a characteristic syphilitic endarteritis. The suggestion has been made (Kolmer) that this difference of localization may be determined by the type of occupation or activity pursued by the patient in the first few months following the acquirement of the infection. The normal wear and tear of organs occasions constantly microscopical regions of degeneration and necrobiosis. Under normal conditions repair is successful and uneventful. The organ or organ-system most affected will be that on which the worker's occupation puts especial strain. In the case of hard physical labor the vascular

¹ Goadby, and Timbrell: *Lancet*, July 1 and 8, 1922.

system will receive the brunt; in the case of protracted mental effort, the central nervous system. In the presence of incipient syphilitic invasion, the microscopical regions of degeneration and necrobiosis serve as *loci resistantiæ minoris*, favoring the arrest of *Treponema pallidum* and consequently determining neurosyphilis or cardiovascular syphilis. This is not the only proffered explanation for the neurotropism or angiotropism of syphilis and the focal lesions of normal wear and tear may not in the narrow sense of the word be traumatic in nature.

In tertiary syphilis gummata have frequently developed at sites which had experienced mechanical injury.

The subject of trauma should not be left without some reference to the danger involved by submitting oneself to treatment at the hands of non-medical practitioners of massage, mechano-therapy and "adjustments." Careless manipulation of malignant pustule or of boils and furuncles have resulted in wide-spread anthrax infection or staphylococcal "blood poisoning" respectively. Even such an innocent thing as squeezing an acne pustule on the lip has resulted fatally. Swollen lymph nodes in the neck, axilla, groin or elsewhere should be treated with particular respect. They should be left strictly alone except for the minimum palpation necessary for diagnosis by a properly trained physician or dentist as the case may require. The ignorant manipulation of a stiff, painful or swollen joint has called forth a general blood infection with secondary localization in other joints or endocardium, or generalized tuberculosis. Not the least of the harm done by adherents of some pseudo-medical cults is of this nature. The meaning of *primum non nocere* is unknown to them, either in Latin or in English.

It is well known that trauma predisposes to some infections of the oral cavity. It is legitimate to regard calculi in the ducts of the salivary glands as traumatic agents, and this condition predisposes these glands to infection. It is possible to regard the calculus which is deposited on the teeth similarly as predisposing to gingival and periodontal infection. The trauma resulting from imperfect occlusion is widely reputed as a potent factor in the origin and persistence of periodontal infection. In the same category should be placed bridges which improperly load the abutment teeth; partial dentures can exert essentially the same effect. Actinomycotic, syphilitic and tubercular infections have been implanted on the tongue and mucous surface of the cheeks when the tongue or cheek is bitten, in sockets following extraction, or in periodontal tissue chronically injured by loose abutments. The generalization

of previously local infections has unquestionably in rare instances been occasioned by dental extractions.

To summarize the relation of trauma to infection, it may be said that the mechanical injury may serve to introduce the micro-organism into the body of the host; it also tends to lower the local resistance so that infectious agencies may successfully implant or localize themselves; it may reawaken a latent infection to activity; it may serve to determine a general distribution of the microbes throughout the circulation, from what had previously been a localized lesion.

11. **Temperature Changes.**—Exposure to chilling is a predisposing factor to infections of the respiratory tract. Mudd and Grant¹ have contributed to our understanding of the mechanism involved. By a thermo-galvanometric method they showed that the temperature of the skin and pharyngeal mucosa fell with chilling of distant areas of the body surface and rose again on rewrapping the subject. The fall is a result of vasoconstriction and the rise of vasodilation. Chilling of the body surface causes reflex vasoconstriction and ischemia in the mucosæ of the palate, faucial tonsils, oropharynx and nasopharynx. Cutaneous chilling also increased the volume of respiratory exchange, which increase usually caused a drop in the temperature of the mucosa. These data would seem to indicate "that the ischemia incident on cutaneous chilling, by decreasing cell respiration, or by retarding the removal of the products of cell metabolism, or by increasing the permeability of the epithelial cell surfaces to bacterial products, or by decreasing the local supply of specific antibodies, or by altering the media in the tonsillar crypts and folds of the pharyngeal mucosa in which the bacteria are living, or by a combination of these factors, might . . . so disturb the equilibrium between host and parasite as to excite infection."

Cope² on duty with British troops in Mesopotamia during the World War described an acute necrotic parotitis. It involved an invasion of streptococci and staphylococci ascending Stenson's duct. The essential prerequisite, however, was considered a desiccation of the mouth sequent to constant high external temperatures, *e. g.*, 120° F. in the shade. This mechanism is similar to that which at times holds for the occasional cases of parotitis following major abdominal operations.³

12. **Climate.**—This term includes a large group of factors and adequate treatment of the subject would require their separation

¹ Jour. Med. Res., 1919, **40**, 53.

² Brit. Jour. Surg., 1919, **7**, 130.

³ Deaver: Ann. Surg., 1919, **69**, 128. Collins: Surg., Gynec. and Obst., 1919, **28**, 404.

and their separate evaluation. Many infections exhibit regular seasonal fluctuations. Pneumonia and typhoid fever are such.¹

The geographical distribution of arthropod hosts necessary for the transmission of a microorganism to man is largely determined by the climate. If a district be free of anopheline or *Stegomyia* mosquitoes, it will of course be free from malaria and yellow fever. The geographical distribution of the various trypanosomiasis (except dourine) corresponds with the distribution of the transmitting diptera or fleas. Arthropod-borne infections can naturally only be prevalent during the season at which the arthropod in question is active and is present in greatest numbers.

Infant mortality in congested districts rises to its height in the summer months. Gastro-intestinal complaints predominate and probably are caused by the high bacterial content of the milk. Cutting down the bacterial multiplication by refrigerating the milk has cut down in a striking manner this morbidity and mortality.

Guthrie² noted a seasonal variation in the incidence of ulcerative stomatitis, reaching a maximum in January and February. This fact he refers to varying dietary conditions. Reckord and Baker³ likewise found a seasonal variation in the incidence of ulcerative stomatitis, reaching a maximum in February. They were working upon young adults in the latitude of Massachusetts while Guthrie's observations were based upon infants and children in the latitude of New Orleans.

13. Occupation.—The factors included in this term act in several ways. Trades in which phosphorus or mercury are used predispose the workers to phosphorus necrosis of the mandible or to mercurial stomatitis. Both these conditions are characterized by the presence of infection. Reference is made to them in the section on intoxications as predisposing factors. Dust is one of the most serious hazards. It is important in pulmonary infections, *e. g.*, tuberculosis and miner's phthisis. "Wool-sorter's" disease is a pulmonary form of anthrax. The cutaneous form appears also among workers from scratching or rubbing the endospores into the skin. Anthrax infection is a hazard to which are exposed those whose occupation calls for the handling of skins, hides, bristles, hair, etc. Tetanus occurs among the makers of rope from fecal contamination of the jute used. Agricultural workers are predisposed to certain infections which are common to man and the domestic animals, *e. g.*,

¹ Huntington: *Civilization and Climate, and World Power and Evolution*, Yale Univ. Press. Rosenau: *Scient. Monthly*, 1925, **20**, 192.

² *Jour. Am. Med. Assn.*, 1920, **75**, 1245.

³ *Ibid.*, 1620.

anthrax and actinomycosis. This latter condition may appear in the skin, especially about the face and neck; or in a pulmonary form from inhalation or more rarely in an intestinal form from ingestion. Glanders often with a nasal localization should be mentioned here, particularly affecting those handling horses.

Glass-blowers and players of wind instruments are subject to infection of the ducts and parenchyma of the salivary glands. The heightened, intra-oral air pressure tends to dilate the ducts. The bacteria entering with the saliva and food débris, institute the infection.

Hook-worm disease has been noticed as occurring among coal miners, particularly in Wales and England.

Dentists in the performance of their work at times acquire infection. Pathogenic bacteria always or almost always inhabit the mouth. The chisel or bur slipping and cutting the finger of the operator occasionally is followed by more or less severe suppuration, causing loss of time from the office and even death. Preëxistent cuts or scratches, such as often are seen around the finger-nail, may afford wide-open portals for the microorganisms of the saliva. Syphilitic infection has been innocently acquired in these ways. One case of Vincent's infection of the finger has been reported as the result of being bitten by an insane patient suffering from Vincent's stomatitis. A dentist was not the victim in this case but the inference is obvious. Ocular and conjunctival involvement has resulted from the patient's saliva or fragments of calculus flying into the dentist's eye. All accidents of this nature should be immediately and thoroughly attended. Bridgman¹ reported one instance in which mercurial salivation was attributed to the habit of mixing amalgams in the palm of the hand. Although no stomatitis is mentioned, it is unlikely that the salivation could exist without there being some sign of the stomatitis. Finally it must not be forgotten that the dentist must work close to the mouth and nose of the patient. This relation would favor the transference of any of the "respiratory" infections to the operator.

14. **Diet.**—Food can predispose to infection in many ways. It can serve as a vehicle for pathogenic bacteria. Botulism from the consumption of contaminated sausages or ripe olives, and the paratyphoid and enteritic infections are common examples in this category. A typhoid patient or carrier may contaminate milk and other articles of diet. Diphtheria, scarlet fever, septic sore

¹ Brit. Jour. Dent. Sci., 1873, 16, 548, and 1874, 17, 43.

throat and similar affections may be transmitted in this way. Other parasites than bacteria can be transferred mechanically by foods, *e. g.*, intestinal worms and *Trichinella*. Uncooked or insufficiently cooked or canned foods are often incriminated. The practical problem is difficult.¹ Current views on nutrition tend to emphasize the desirability of eating some uncooked foods while economic and social conditions require canned goods on a large scale.

Inanition, from whatever cause, starvation, quantitatively or qualitatively inadequate diets, unbalanced diets, diets with deficient content in one or another of the several "vitamins;" any one or all of these or other circumstances, if continued long enough might be expected *a priori* to decrease one's resistance to one or another of the infections. K. F. Meyer² "found that guinea-pigs, which are normally immune to the effects of typhoid bacilli given by mouth, will become infected if they receive the germs while they are on a diet of bread and milk." Ficker³ found, particularly in rabbits, that starvation favored the passage of bacteria through the intestinal mucosa. Large meals rich in fat,⁴ it has been shown experimentally, likewise increase the permeability of the intestinal mucosa to bacteria. This fact together with the naturally greater permeability of the intestinal mucosa of children and infants, suggests itself as a factor to be considered in the intolerance some infants manifest to fats in their diet and in the gastro-intestinal disturbances seen in such cases. Koch and Smith⁵ found a lowering in the amount of complement or alexin in the blood serum of starving guinea-pigs. As we shall see later, complement or alexin is important in the defense of the animal against infection. It is well known that diet influences intestinal fermentation and putrefaction. These processes are expressions of bacterial activity and are more or less seriously detrimental to the macroörganism. An instructive instance of the significance of diet in the problem of infection is reported by Webster and Pritchett.⁶ Two sets of white mice of the same stock were fed on two standard diets. The first diet has long been in satisfactory use at the Rockefeller Institute. It consists of baker's bread and pasteurized milk (Grade B) supplemented by an oatmeal and buckwheat mixture and dog biscuit. The second

¹ Jordan, E. O.: Food Poisoning, Univ. Chicago Press, 1917, pp. 107 *et seq.*

² Quoted by Alvarez: *Physiol. Rev.*, 1924, **4**, 382.

³ *Arch. f. Hyg.*, 1905, **52**, 179.

⁴ Desoubry and Porcher: *Compt. rend. Soc. de biol.*, 1895, **47**, 101.

⁵ *Proc. Soc. Exper. Biol. and Med.*, 1924, **21**, 366.

⁶ *Jour. Exper. Med.*, 1924, **40**, 397.

set was fed on a McCollum complete diet (whole wheat bread, casein, milk powder, NaCl, CaCO₃ and butter fat). The two sets of mice apparently thrived equally well. There were no manifestations attributable to dietary deficiencies as now recognized. Nevertheless from the stand-point of resistance to infection the existence of a profound difference is indisputable. The animals fed on the McCollum diet exhibited a far greater resistance to the bacillus of mouse typhoid and to botulinus toxin, than did the animals fed on the regular Rockefeller Institute diet.

Great interest at the present time has centered about the relation of those peculiar, accessory food substances, probably most distinctively designated by the term *vitamins*. The disturbed metabolic or nutritional condition which results from an inadequate vitamin-content in the diet, is known as an avitaminosis. The nature of this condition varies with the vitamin at fault and often entails a decrease in the normal resistance offered to some infections. The effect of what probably today would be interpreted as an increased susceptibility due to vitamin deficiency was some time ago reported by Hankin. Rats are under normal conditions very refractory to anthrax. One-half of a new-born litter were fed with the ordinary mixed food with relatively large amounts of meat. This group proved to be relatively insusceptible. The other half were fed with bread and milk and all readily succumbed to experimental anthrax infection.

Three distinct vitamins or perhaps rather three distinct groups of vitamins have been recognized; fat-soluble A, water-soluble B, and water-soluble C. The best-known infection associated with a deficiency in the fat-soluble A, is probably the xerophthalmia of man and some other mammals. These ocular infections yield readily when adequate fat-soluble A is restored to the diet, *e. g.*, by cod liver oil. In the words of Sherman and Smith¹ this "eye condition undoubtedly involves infection and in this sense is not purely a deficiency disease, yet it is essentially so, inasmuch as the dietary deficiency so enormously increases the susceptibility of the animal to the infection as practically to determine the occurrence of the disease. It is also very significant that without any other treatment whatever the eye disease, if not too far advanced, usually disappears quickly when the animal is given any food containing a sufficient amount of the vitamin A." Increased susceptibility to infections of the respiratory system has similarly been

¹ The Vitamins, 1922, p. 189.

reported by McCollum and by Drummond. Daniels, Armstrong and Hutton¹ find evidence indicating that fat-soluble A plays an important role in the resistance offered to pyogenic bacteria. The rats used in their experiments contracted a nasal sinusitis and some of them also showed abscesses on the tongue near its base. Cramer and Kingsbury² concluded that a diet deficient in fat-soluble A impairs the local tissue defenses of the intestine, eyes, and lungs. Such deficiency, however, does not appreciably diminish the efficiency of the general humoral defenses as indicated by agglutinin formation.

This latter fact has been observed independently a number of times. Werkman³ fed rats and rabbits on rations deficient in fat-soluble A until the avitaminosis was advanced. Immunization was instituted, using appropriate controls. No difference was demonstrable between the experimental and the control animals in respect to their ability to form agglutinins, precipitins, hemolysins and bacteriolysins. Likewise the absence of water-soluble B from the diet of rats did not affect the elaboration of these antibodies. The production of agglutinins for the typhoid bacillus was also found in pigeons fed on a diet of inadequate water-soluble B content. Werkman also observed no differences from normal in the normal opsonin content of the blood of the rat lacking fat-soluble A or water-soluble B, when he used *in vitro* methods. The same absence of differences was the case in the rabbit lacking water-soluble A. The same results were obtained when *in vitro* determinations were made of specific immune opsonins. When however the determinations were made *in vivo* the results were slightly in favor of the control animal, *i. e.*, a factor in the lowering of the defense against infection seemed to be a lowering of the opsonic power. Werkman remarked that this effect might result from differences in the body temperatures. He sometimes found the temperature of the animal with the avitaminosis 10° F. below that of the controls. This observation is of interest in connections with those of Findlay given below. Besides this drop in body temperature, Werkman also noted a decrease in the blood platelets and depressed leukocyte and erythrocyte counts in rats lacking vitamin A or B.

Essentially similar results have been reported by Werkman, Nelson and Fulmer⁴ in the case of vitamin C deficiency in guinea-pigs. There occurs a definite though not marked break in the resistance of these animals to the pneumococcus and *B. anthracis*.

¹ Jour. Am. Med. Assn., 1923, **81**, 828.

² Brit. Jour. Exper. Path., 1924, **5**, 300.

³ Abstr. Bacteriol., 1923, **7**, 26.

⁴ Jour. Infect. Dis., 1924, **34**, 447.

The rectal temperature was slightly lower in the avitaminotic guinea-pigs than in the controls before the injection of bacteria. No difference in the ability to produce agglutinins for the typhoid bacillus were revealed between the experimental and the control animals. Likewise the phagocytic mechanism seems unimpaired as a result of vitamin C deprivation.

The relation of deprivation of vitamin B to bacterial infection has been carefully studied by Findlay.¹ Pigeons fed on such diets become susceptible to infection with the pneumococcus and meningococcus organisms to which they are naturally highly resistant. Increased susceptibility was also apparent toward *Bacillus coli* and *B. enteritidis* (Gaertner). This reduction in natural immunity only becomes marked when the cloacal temperature drops to 40° C. or below (normally it is about 41° to 43° C). "The lowering of the body temperature appears to decrease the resistance by (a) *facilitating the growth of the invading organism*, (b) reducing the leukocytic response to the infection, and (c) reducing the bactericidal power of the leukocytic exudate."

The relation of water-soluble C or antiscorbutic factor to infection has also been studied by Findlay.² Guinea-pigs deprived of vitamin C succumb to a smaller infecting dose of pneumococci, *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Bacillus coli* than do animals fed on a complete diet. Essentially the same observations had been made by Abels.³ This lowered resistance may in part be due to degenerative changes and feeble leukoblastic response seen in the bone-marrow in guinea-pigs with chronic scurvy (Findlay). Schilf⁴ found that tubercular guinea-pigs, deprived of vitamin C, completely or partially lost their cutaneous reactivity to tuberculin. In this case as in Findlay's work on vitamin B deprivation, the tubercular animals without vitamin C showed a subnormal body temperature while the tubercular controls in normal diet were febrile. Leichtentritt⁵ prolonged the life of tubercular guinea-pigs by feeding them large amounts of neutralized lemon juice, *i. e.*, by enriching their rations with vitamin C. As a rather unexpected phenomenon, the complement content of scorbutic guinea-pigs according to Koch and Smith⁶ rises during scurvy. *A priori* this would indicate a rise in the defensive resources. It has been mentioned above that

¹ Lancet, 1922, **202**, 714; Jour. Path. and Bacteriol., 1923, **26**, 485.

² Jour. Path. and Bacteriol., 1923, **26**, 11.

³ Wien. klin. Wchnschr., 1920, **33**, 899.

⁴ Centralbl. f. Bakteriol., 1924, **91**, 512.

⁵ Deutsch. med. Wchnschr., 1924, **50**, 672.

⁶ Proc. Soc. Exper. Biol. and Med., 1924, **21**, 366.

antibody production as judged by the quantity of antibodies in the blood is not materially altered by vitamin deficiency.

A classical and prominent symptom in scurvy is a more or less severe gingivitis or stomatitis. There is in prolonged cases destruction of tissue, exposure of the roots, loosening and exfoliation of the teeth. All this is accompanied and complicated by infection implanted on these oral parts. The microorganisms are varied with Vincent's types predominating.

The constancy and importance of the inflammatory reaction in infections has been pointed out. It seems to be modified by dietary and nutritional influences. Arkwright and Zilva¹ report that the maintenance of the same weight for about fourteen days in a young guinea-pig of 250 gr. has a decided effect in causing the inflammatory reaction to the intra- or subcutaneous injection of diphtheria toxin or living bacilli, to be smaller than it is in the growing animal. A fall in weight of 50 gr. in five or six days, whether due to general reduction in the diet or to a deficiency of vitamin C, makes the inflammatory reaction less than half the size exhibited by the control. The reaction becomes still smaller with further reduction in weight. The edematous effusion may become negligible, and there is less congestion and apparently less cellular exudate in the local reaction of reduced animals following the injection. Cramer, Drew and Mottram² had already noticed a marked atrophy of the lymphoid tissue in the absence of vitamins. Lymphocytes apparently act as detoxicating agents and are found in the more chronic types of inflammatory exudates. In view of the defensive function of inflammation it is easy to understand that interference with its development may be fraught with danger to the host.

This review of the factors predisposing to infection is far from being complete. It is introduced primarily to show how complicated is the problem. While we cannot have an infectious disease without the microorganism, certain supplementary factors must coöperate before the actual infection can be consummated. The microorganism can only become effectively operative in a favorable environment and under favorable conditions. For instance we cannot have dental caries without bacteria, but we can have the bacteria without having the dental caries. In this condition the crux of the question of susceptibility and immunity seems to lie in the environing, attendant, predisposing circumstances. Simi-

¹ Jour. Path. and Bacteriol., 1924, 27, 346.

² Lancet, 1921, i, 963.

larly in periodontal disease, we encounter in one patient marked alveolar atrophy plus a severe pyogenic infection, while in another patient although the same degree of alveolar atrophy be present, the infectious phase is very trivial and practically negligible.

While all the factors mentioned in the above paragraphs have been at least surmised from the earliest days of bacteriology, the vast majority of investigations have either ignored them or have paid only slight and incidental attention to them. In recent years, with our knowledge of the exciting agents relatively satisfactory, the predisposing factors are being studied as central problems in research. This attitude is urgent for as Pearl states¹ “. . . for many causes of death a vast lot needs to be added to our knowledge of etiology in the broadest sense . . .” For a complete natural history of infection and for the satisfactory rational control of infections, it is necessary to recognize and to evaluate *all* the factors.

¹ Biology of Death, Philadelphia, 1922.

CHAPTER X.

TYPES OF INFECTION.

It is customary to present some such outline as that which follows, as a classification of the fundamental types of infection.

A. Local.

1. Circumscribed.
2. Diffuse.

B. General.

1. Toxemia.
1. Bacteriemia.
 - (a) Pyemia.
 - (b) Septicemia.

LOCAL INFECTION.

Abstractly or formally considered, a local infection is characterized by the fact that the microorganisms are confined to a small region of the body of the host and that the reaction of the tissues is confined to the immediate neighborhood of the microorganisms. In the case of a general infection, the reaction on the part of the host is not limited to the site where the parasite is present but involves more or less intensely the macroorganism as a whole. These definitions, as is also generally the case with biological definitions, are only partly true. As a matter of fact the difference between a local and a general infection is only relative. The distinction is probably useful clinically but in all likelihood every so-called local infection calls forth in some degree a general response. The organism as a whole is probably always involved. If the systemic involvement be trivial or unrecognizable clinically one speaks of a *local* infection. If the involvement be clinically obvious or significant, one speaks of a general infection. Many general infections occur as sequels to frank local infections: for example, anthrax septicemia following malignant pustule or pyemias following boils or carbuncles.

These three conditions, malignant pustule, boils and carbuncles, serve to illustrate *circumscribed* local infections. The tissues involved in the reaction immediately around the bacteria are to the naked eye fairly sharply demarcated from the surrounding

normal tissues. Lupus and some phlegmonous processes may serve as illustrations of diffuse local infections, where the demarcation between healthy and affected tissue is obscure.

GENERAL INFECTION.

Of the general infections, the toxemias are classically exemplified by diphtheria and tetanus. In these conditions, the microorganisms are confined usually to a very limited area or region. The general symptoms are due to the absorption and distribution of the soluble poisons elaborated by the bacteria at these sites. The bacteria themselves never maintain themselves in the blood stream, except in rare instances shortly before death of the patient.

In the bacteriemias the bacteria are able to survive and even to multiply in the blood stream. Certain protozoan infections, *e. g.*, malaria and the trypanosomiasis, also illustrate essentially the same condition. In case the bacteria, distributed through the blood stream, become localized at various points, *e. g.*, heart muscle, liver, kidney, or brain, and at these sites of secondary localization attract neutrophilic polymorphonuclear leukocytes in appreciable numbers, this type of infection is known as pyemia. In other words, pyemia is the condition in which pyogenic bacteria are in the circulating blood and in which multiple abscesses develop where these bacteria become arrested in their passage through the organs. *Staphylococcus aureus* frequently is responsible for pyemias.

To cases in which we have a bacteremia but in which pyemia does not develop, the term *septicemia* may legitimately be applied. In this sense generalized anthrax and Asiatic cholera are septicemias. At certain stages in its course typhoid is a septicemia, and meningococci have been isolated by blood culture before the characteristic, nervous symptoms appeared. In streptococcal endocarditis and in the generalized streptococcemia of severe puerperal infection, there exists a septicemia. In the latter condition the overwhelming nature of the invasion prevents the appearance of a pyemia, which always represents a certain degree of resistance on the part of the patient.

FULMINATING, ACUTE, SUBACUTE, AND CHRONIC INFECTIONS.

Infections are designated according to the intensity of their course as fulminating, acute, subacute, and chronic. Fulminating infections are of the utmost severity. They develop with lightning-

like suddenness (whence the name) and overwhelm the patient apparently before the defenses can be mobilized. Fulminating cases are often seen at the height of epidemics, *e. g.*, plague, typhus, cholera, influenza, diphtheria, scarlet fever, and also in puerperal septicemia. They may occur though with greatest rarity, following dental extractions. The acute infections include such conspicuous diseases as pneumonia, typhoid, cholera, bubonic plague, smallpox, mumps, cerebrospinal meningitis, anterior poliomyelitis, tetanus, measles, diphtheria, scarlet fever, whooping cough, chicken-pox, etc. The symptoms are usually distinctive and characteristic. The classical division of the course of such infections into an incubation period, prodromal symptoms, fastigium, defervescence and convalescence, is often easily made. The phenomenon of *crisis*, when the time at which the disease takes a decided turn for worse or better can be rather exactly recognized, is sometimes exhibited in these acute infections, as in pneumonia. The interval between the onset of the infection and the eradication of the parasite from the body or the death of the patient, is relatively short compared with the same interval in the chronic infections. This characteristic is possibly as good a distinction between acute and chronic infections as can be drawn. In fact this is implied in the word *chronic* itself which is from the Greek word meaning "time." The severity of the symptoms or the urgency of the condition of the patient are not peculiar to acute infections.

Many infections begin as acute conditions and later assume a chronic course. A well-known instance is appendicitis. Subsidence of an acute attack does not mean the elimination of infection. Rather the disease becomes chronic, with a tendency to acute exacerbations. Some cases of infective cholecystitis offer a parallel history. Gonorrhea is notorious in this respect. The secondary symptoms of syphilis, the cutaneous and mucous eruptions, the sore throat, the swelling of the regional lymph nodes, the malaise, the fever and sensations of chilliness, are certainly suggestive of an acute condition, but commonly the duration of this infection is a matter of years with long symptomless periods. Tuberculosis, leprosy, actinomycosis, malaria are sharply defined, specific infections which habitually run a very slow course. Lying between the frankly chronic and the frankly acute infections, are those termed *subacute*.

The subject of chronic infections involves the consideration of subinfection, latent infection and focal infection.

LATENT INFECTION AND SUBINFECTION.

A pathogenic microörganism may reside in the body of the host for a relatively long time without exerting any recognizable damage or determining any recognizable reaction. To this condition the term "latent infection" may appropriately be applied. At a later period, under circumstances of which we know little, this focus may awaken to activity and occasion clinical symptoms. A well-known instance is sometimes afforded by tetanus in which the disease may suddenly develop months after the wound through which the microörganism presumably was introduced, had healed. A related condition was observed in the surgical experience of the World War. This was particularly the case when bone tissue was involved. Apparently the microörganisms had been introduced when the punctured or lacerated wound was inflicted and would remain latent or dormant for apparently an indefinite period. It was often thought desirable in such cases, long after the wound had healed, and when the strength of the patient warranted, to perform a supplementary operation to correct a functional fault or or cosmetic considerations. In some such cases in which the surgical technic was above reproach, operative interference would be followed by the development in the affected tissues of gas gangrene or some related infection. Apparently the reopening of the wound so lowered the vitality of the tissues, so disturbed the equilibrium between host and parasite, that the parasite got the upper hand long enough to call forth a clinically recognizable reaction.

Latency is frequently seen in tubercular infection. The tubercle bacilli may persist in some arrested or "healed" lesion for years, to all appearances completely inert. Then for some obvious or obscure reason it may suddenly resume activity and occasion a serious or even fatal invasion.

The terminal infections, carrying off the patient after he has been worn out by some other disease, may be regarded as sequels to latent infection. As will be pointed out in the discussion of the natural defenses against infection and as will be further developed below in the section on subinfection, bacteria, usually of low virulence or ordinarily considered saprophytes, are constantly entering the body from the respiratory and alimentary tracts. While the individual is in health these bacteria are destroyed without any determinable ill effects. When the individual is below par, or when for any reason the number of incoming bacteria exceeds a

liminal dose, these bacteria may occasion all grades of disturbance from what we shall describe below as subinfection to the fatal, terminal or agonal infections. The presence of living bacteria in the tissues, which ordinarily are destroyed but which may do harm under certain conditions, may logically be considered an instance of *latent infection*.

It is difficult practically to draw the line between latent infection and *subinfection*. In subinfection during the entire period of its persistence some damage is actually being done to the host. The amount of damage suffered at a given time is so small that no recognizable clinical symptoms may appear until long after the condition has been in existence. It is the cumulative effect of the continuous drain upon the host that is so serious.

The concept of subinfection we owe to Adam¹ and its nature and significance may be best appreciated by reference to its historical development. One of the problems which in the early days of bacteriology seemed most urgent, was the question of the sterility of normal, healthy tissues. As a typical illustration of the investigation of this problem reference may be made to the publication of Mott and Horsley.² At this time it appears, at least seven authors had expressed themselves in print on this topic. Five concluded that bacteria do exist in healthy living tissues, and Mott and Horsley were in agreement with these; while two had secured negative results. Bizzozero³ and Ribbert⁴ both found bacteria in healthy mesenteric glands. Ruffer⁵ found enormous numbers of microbes in Peyer's patches, the tonsil and the peribronchial lymph nodes of the healthy rabbit. Wurtz⁶ found evidence that in the last moments of life, when death is due to cold, intestinal bacteria can penetrate into the peritoneal cavity and into the blood. It was suggested that the mechanism involved was that the cold determined congestive lesions of the intestine through which the bacteria entered. Nocard⁷ reported that the blood serum of fasting animals is nearly always sterile as also, to a less extent, is their chyle. After feeding, however, microbes were present in the chyle in considerable numbers, being less where lean food had been taken (see Ravenel *infra*) while after a meal of fatty foods they formed innumerable colonies.

¹ Jour. Am. Med. Assn., 1899, **33**, 1509, 1572; Brit. Med. Jour., 1914, i, 177.

² Jour. Physiol., 1882, **3**, 188.

³ Centralbl. f. d. med. Wiss., vol. **23**, 491.

⁴ Deutsch. med. Wehnschr., 1885, **3**, 197.

⁵ Brit. Med. Jour., 1890, ii, 491.

⁶ Compt. rend. Soc. de biol., 1892, **4**, 992.

⁷ Compt. rend. Soc. de biol., February 9, 1895.

These observations were confirmed by Porchard and Desoubry.¹ Adami² observed in sections of healthy mesenteric lymphatic nodes of man, in general, definite bacteria undergoing degeneration. Nichols (per Adami, 1899) demonstrated in the mesentery of normal rabbits relatively numerous bacteria in various stages of disintegration. Ficker³ and Hilgermann⁴ showed that bacteria can more easily penetrate the intestinal mucosa in young than in older animals. Ravenel⁵ has pointed out that the penetration of tubercle bacilli through the intestinal mucosa of dogs is facilitated during the digestion of fats. If the food was free from fats, few or no bacteria could be found in the mesenteric lymph nodes. Besredka⁶ reports that the administration of ox-bile can render rabbits susceptible to typhoid and cholera infection when the corresponding bacteria are mixed with the food. Without the bile, this method is unsuccessful in establishing infection. The ox-bile stimulates the secretion of the rabbit's own bile and the combined action results in desquamation of the intestinal mucosa.

Other references to the penetration of bacteria through the intestinal mucosa and also through the lining of the respiratory channels are given in the section of this book, on the natural defenses against infection. Similar instances could easily be multiplied. Enough has been given to show that under a great variety of conditions, normal as well as more or less abnormal, living bacteria are constantly gaining access to the tissues of the living animal. Under ordinary circumstances and continuing for many years, the defensive powers of the body are easily able to destroy these bacteria. This condition can be stated in brief as follows: The tissues of the living, normal body are not *invariably actually* sterile. The distinguishing characteristic of healthy in contrast with diseased tissues from this stand-point, is that in the former case (healthy) the tissues are *potentially* sterile. Even though the bacteria be rapidly entering, they are being destroyed with at least equal rapidity. This constant entrance and destruction of bacteria is the condition of subinfection. When with recurrent invasion the tissues become worn out, chronic or acute infection may supervene. Other things being equal, it is conceivable that the more bacteria which enter in a given time, the more rapidly will the tissues be

¹ Med. Weekly, 1895, p. 227.

² Loc. cit., 1899, 33, 1511.

³ Arch. f. Hyg., 1905, 52, 179.

⁴ Ibid., 1906, 54, 335.

⁵ Am. Jour. Med. Sci., 1907, 134, 469.

⁶ Paris méd., 1922, 43, 460.

worn out. In subinfection with the destruction of the invading bacteria, the liberation of their "toxins" causes a poisoning of the cells immediately around them. The accumulative effect of these "toxins," either locally or at a distance, *e. g.*, upon the liver cells, is to bring about the death of certain cells and replacement with fibrous tissue.

Subinfection may account for certain cases of "autointoxication"¹ and may contribute to certain of the "degenerative" diseases, affecting the kidneys, arterial walls, cardiac musculature, which appear with increasing frequency from middle age on. It will become obvious in our discussion of focal infection in Part III, that focal infections can be considered subinfections. Chronic tonsillar, periapical, gingival and periodontal infection can continue for years. The patient may be unaware of the presence of infection. The bacteria which continuously or periodically invade the body from these foci or the toxic products which diffuse out from these foci may be destroyed—but their effects accumulating through years cannot but be harmful to the host. When for any reason the local resistance of an organ or part or the general resistance be lowered, some of the bacteria entering from these foci may survive and serve to establish secondary localizations, as *e. g.*, in the heart-valve leaflets or joints. The infectious foci in themselves acting as subinfections, tend to lower the resistance of the body and in this way a vicious circle is established.

MIXED INFECTION.

Mixed infections are where two or more microörganisms are etiologically concerned at the same time. These conditions may be mixed from the start or they may be initiated by one parasite which prepares the way for other species to participate as secondary invaders. In this latter circumstance one speaks of *secondary* infection. In mixed infections, primary or secondary, little is known of the relative parts played by the organisms concerned. We know, for example, in pulmonary phthisis that the tubercle bacillus is the primary agent: and there is evidence that the cavity formation and the particularly disturbing febrile symptoms are attributable to the later invasion of the diseased tissue by pyogenic staphylococci, streptococci, pneumococci, tetracocci, *Micrococcus catarrhalis* Friedländer's bacillus, etc. Similarly, putrid or gangrenous pro-

¹ Alvarez: *Physiol., Rev.*, 1924, 4, 382.

cesses may occur in tubercular lungs, due not directly to the tubercle bacillus, but to the secondary arrival of Vincent's or Castellani's spirochete and fusiform bacillus. The possibility of unfortunate secondary infections occurring is an obvious reason for the routine maintenance of oral health in the tubercular.

Mixed infections, or at least the association of more than one potentially pathogenic species, are common in the more chronic or obscure types of respiratory and alimentary affections. There is no general agreement as to the cause of the common cold in the head, coryza. In this condition habitually several species of pathogenic types are found, *e. g.*, pneumococci, streptococci, *Micrococcus catarrhalis*, influenza bacilli, etc. At times definite improvement seems to follow the use of vaccins into which these microorganisms have been incorporated. The same remarks are applicable in general to the chronic infections of the accessory nasal sinuses. Several, and often many, bacterial species can be isolated not only from the surface and crypts, but also from the lymphoid substance itself of chronically inflamed tonsils. Likewise the bronchial flora of some asthmatic conditions is mixed.

In the oral cavity, mixed floras of the most varied nature are always encountered in gingivitis and "pyorrhea" pockets. Unless we accept the view of those (Tunncliffe and others) who believe the fusiform bacillus and the Vincent's spirochete to be not distinct species but rather two stages in the life cycle of a single species, then the constant symbiosis of these microorganisms in the various forms of Vincent's infection of the oral and pharyngeal cavities, affords us probably the most striking instance of a mixed infection.

As will be seen in Part III, several bacterial species are often associated in the infections of the dental pulp and periapical tissues.

In the obscurer types of intestinal infections, commonly several species of microorganisms are found and are equally the subjects of suspicion. In the peritonitis following intestinal perforation, mixed infections are the rule, *e. g.*, streptococci and colon bacilli. This holds even when the perforation is attributable to an entirely different species, *e. g.*, the typhoid or tubercle bacillus.

Tetanus affords an illustration of the occasional importance of mixed infections. It seems that the tetanus bacilli are ineffective until and unless the ground has been prepared for their activities by common pyogenic species.

Attempts have been made to classify mixed or secondary infections. For example the following scheme is based on one offered by Babes and Cornil.

1. Association of different varieties of the same bacterial species: *Staphylococcus pyogenes aureus* and *albus*, human and bovine types of tubercle bacilli.

2. Association of two closely related pathogenic bacteria, *e. g.*, streptococci and staphylococci.

3. Association of bacterial species which are not biologically or morphologically closely related, *e. g.*, Vincent's spirochete and fusiform bacillus, streptococci and diphtheria bacilli, the anaërobic acne bacillus of Unna, Sabourand and Gilchrist and *Staphylococcus pyogenes albus*.

4. Association of bacteria with protozoa (amoebæ or trypanosomes) or fungi.

5. Association of different protozoa, *e. g.*, the parasite of tertian and quartan malarial fevers.

6. Association of pathogenic bacteria with species which usually are saprophytic but which under these conditions assume pathogenic properties, *e. g.*, putrefactive organisms in lesions caused by streptococci or diphtheria bacilli.

In the mixed and secondary infections discussed above, we do not include the mere synchronous but unrelated (or very indefinitely or questionably related) presence of two distinct infections in the same individual. For instance an individual may be suffering at the same time with syphilis and gonorrhea, or tuberculosis and malaria, or typhoid and trypanosomiasis. The term *multiple* infection would seem appropriate for this condition.

FOCAL INFECTION.

The subject of focal infection is treated in Part III.

CHAPTER XI.

HOW DO BACTERIA PRODUCE DISEASE?

WHETHER or not an invading microörganism succeeds in establishing an infection depends upon many factors, some of which probably are not even surmised as yet. Some of the factors are inherent in the parasite and some in the host. Some of the obvious groups of factors are: (1) Number of bacteria gaining access to the host in given unit of time; (2) stage in the life cycle of the microörganism at which it enters the host; (3) the "virulence" of the parasite; (4) the site at which it enters; and (5) the "resistance" of the host.

CONDITIONS DETERMINING INFECTIONS.

Minimum Number of Bacteria Necessary. The relation of the *number of bacteria* to the establishment of infection: "The infecting dose." This subject can be best introduced by reference to a simple analogy. Bloomfield and Felty¹ showed that when an unsuitable, inert, culture medium was employed, a certain minimum quantity, greater than unity, of microörganisms was necessary for inoculation in order to obtain a growth in that medium. For example, using 5 cc of meat extract broth, they found that if they introduced into this a number of streptococci, represented by 160,000 colonies, they would succeed in obtaining a growth in that medium; whereas if they introduced only one-half that number, the medium would become sterile within twenty-four hours. If a favorable medium were substituted for the unsuitable extract broth used in the above experiment, the number of organisms needed for a successful transplantation became very small. Profuse macroscopical growth appeared uniformly within twenty-four hours although the inoculum might be so small that control platings showed no colonies.

The analogy with genuine infections is obvious. At one extreme, 2000 million tubercle bacilli usually fail to establish infection in the albino rat, while very few, possibly 2 or 3 invariably succeed in infecting the guinea-pig. Other species, as man, occupy inter-

¹ Jour. Exper. Med., 1924, 39, 367.

mediate positions; and within narrower limits variations in the minimum infecting dose occur in the several individuals of a species or race and even in the same individual at different times.

Bloomfield and Felty¹ in later work extended their studies from determining the minimum number of organisms needed to establish growth in artificial media onto the field of genuine infection. Progressively diminishing the dosage of hemolytic streptococci, *Staphylococcus aureus* and a colon bacillus they found a more or less critical point below which infection was not initiated in white mice. For example, in one of their experiments with the streptococcus, the mortality was uniformly 100 per cent when the number of bacteria injected was 800 million or more, while the mortality was zero when the number had been reduced to 100 million.

The importance of the number of invading microorganisms for the consummation of infection has been recognized since the earliest days of bacteriology. A hypothesis which plausibly visualizes the interacting forces, has been suggested by Dudley.² "For the sake of argument, suppose that all the conditions are fixed and constant. An individual receives V amount of infective agent per hour. The defensive mechanism can destroy U amount of this infective agent per hour; then we may term V the velocity at which infection is received and U the velocity of destruction of infection. If U is greater than V , it follows that no infection can ever take place so long as the conditions remain unchanged, because the infective agent is being dealt with as fast as it can be received. But if V is greater than U , infection can take place, but only if conditions are in operation long enough. The time required will depend on how much greater V is than U , that is, on $V-U$, which is the amount of the infective agent left undestroyed at the end of each hour, and therefore $V-U$ is the velocity at which infection takes place. The nearer U (the velocity of destruction) approaches V (the velocity of reception), the smaller $V-U$ (the velocity of infection) becomes . . . V will of course vary with the source of infection and its distance, and with the conditions of ventilation and humidity. It is now easy to see that for a subject to collect a M. I. D. (minimum infecting dose) of an infective agent he must remain $\frac{M. I. D.}{V - U}$ hours in the same infective environment."

The Stage in the Life Cycle of the Microorganism at Which it Enters the Host.—The importance of this consideration is obvious in the

¹ Jour. Exper. Med., 1924, 40, 679.

² Medical Research Council Special Report Series No. 75, 1923.

case of the parasites of malaria. The sole infective stage for the mosquito is the gametocyte; for man, the sporozoite. Bacteria, at least in culture, exhibit a less distinctive but genuine cycle. The analogy between the smallest number of microorganisms used as an inoculum, necessary to secure growth in unsuitable medium and the minimum infective dose for a moderately resistant animal has been extended by Bloomfield and Felty to this subject. In order to understand the significance of their work it must be recalled that when bacteria from a culture several days old are inoculated into a tube of culture medium, their number either remains stationary or may be reduced for a period of possibly several hours. This period of lag or latency is succeeded by one of rapidly increasing rate of growth, reaching a maximum and then declining to a zero rate. In one of their experiments, it was found that visible growth in the inoculated medium appeared in twenty-four hours when the inoculum was 1600 bacteria from a rapidly increasing culture, while to produce the same effect with an inoculum from a culture in the period of lag 68,000 bacteria were necessary.

As a plausible counterpart in the case of genuine infection, may be cited the often observed fact that younger cultures (eighteen hours or younger) of streptococci in Rosenow's medium are more effective in producing lesions in rabbits than are older cultures (twenty-four hours or older). The younger culture is presumably in the stage of rapid multiplication whereas in the older culture the period of declining rate has supervened.

This phase of the study has been more particularly studied by Bloomfield and Felty.¹ Distinct differences have been demonstrated between relatively young, rapidly reproducing cultures of bacteria and the same strains during the period of declining reproductive activity in respect to invasive power and pathogenicity. These properties are distinctly more marked in the younger cultures. For example, using the white mouse, they found that a dose of 1,750,000 streptococci was lethal when taken from a culture after five hours' incubation. At the end of twenty-four hours, *i. e.*, nineteen hours later, the mice survived a dose of 6,500,000 from the same flask; while at the end of three days' incubation a dose of 25,500,000 was not fatal.

These differences in pathogenicity according to the stage of activity in which the microorganism was when it gained access to the prospective host, may determine whether or not that host will

¹ Jour. Exper. Med., 1924, 40, 703.

eliminate the parasite with or without *clinical* disease. ". . . Bacteria entering in an inactive growth phase for example dried in dust or perhaps from a chronic carrier, may be disposed of before activity can be resumed, whereas organisms introduced in the stage of active growth, as from a case of acute diseases, may be able to take advantage of a portal of entry."

The Virulence of the Parasite. The term "virulence" is used to denote that complex of properties that determines that a micro-organism is pathogenic. In the absence of this complex the micro-organism is non-pathogenic. Virulence implies two rather distinct groups of properties: (1) Those that enable the microorganism to survive in the macroorganism and (2) those that damage the macro-organism. Some of the factors constituting the second group will be considered at greater length under the headings of toxins, endotoxins, bacterial proteins, hemolysins and leukocidins. Little that is satisfactory is known about the first group although some emphasis has been laid upon the production of certain substances by the bacteria, which counteract or paralyze the defensive forces of the host. The term "aggressins" has been given to such substances. Capsulogenic strains, as of the pneumococcus or anthrax bacillus, are generally considered to be more virulent, *i. e.*, to be better able to survive in the host, than are the corresponding non-capsulogenic strains. Again, it has been repeatedly observed that strains of pneumococci which are soluble in bile are in general more virulent than are pneumococcal strains which are not soluble in bile.¹

It is almost self-evident that an organism to be a parasite must be adapted to the physical, chemical and physico-chemical conditions which it encounters in its prospective host. The nutritive supply furnished by the host must be quantitatively and qualitatively adequate. The temperature, osmotic pressure, hydrogen-ion concentration, and oxygen tension of the environment offered by the macroorganism must be optimal for the growth of the microorganism. The satisfaction of these conditions is an obvious requisite for parasitism.

Important as "virulence" is, it is a very elusive and unstable characteristic. Bacteria morphologically, culturally and biochemically indistinguishable from true diphtheria bacilli are often isolated from throats. They differ, however, in at least the one essential property: They are unable to produce diphtheria toxin, and are non-virulent. The relation of these strains to virulent strains is

¹ Neufeld und Haendel, and Cotani, Truche et Raphael: Monographie de l'inst. Pasteur, 1922.

unknown. Most pathogenic microorganisms lose their virulence when grown for some generations on artificial media. Tubercle bacilli in this way have in the course of years become avirulent even for guinea-pigs. Virulence may be absolutely lost or it may only be lessened. It is sometimes possible for a bacterial strain to regain fully its pristine powers of disease-production. Conditions responsible for the loss of virulence are enumerated in our later discussion of means of active immunization.

Pasteur demonstrated in the case of the rabic virus in the rabbit the enhancement of virulency by repeated passage through a susceptible animal. Similar enhancement in the case of other microorganisms and hosts has since then been repeatedly accomplished. Felton and Dougherty¹ succeeded by an *in vitro* method in raising the virulence of a pneumococcus from practically zero to such a degree that a single diplococcus would kill a mouse.

Spontaneous inexplicable fluctuations in virulence occur. For instance, Nicolle and Cesari² report a strain of pneumococcus which diminished considerably in virulence when kept on ice, but after one year had regained its original degree. This level was maintained for some months when a decline set in ending in total loss of virulence, a condition which has persisted for at least thirteen years. That this observation is not exceptional is likely for a similar cycle has been reported by Flexner and Amoss³ in a form as different from the pneumococcus as the virus of poliomyelitis. The strain in question readily achieved high virulence toward the monkey. This level was maintained for about three years when diminution became apparent. The virulence declined to reach approximately the level of the original human virus. This stage was followed by recovery of its high virulence, accomplished during a sojourn of several years in glycerol. This period of recovered, maximum virulence has persisted for at least four years.

It is well to remember that the degree of virulency does not rise and fall for all species of hosts together. A decrease of virulence for one species of host may leave the virulence for other host-species unaffected; and also increased virulence for a given host-species does not entail increase for other host-species. The virulency of a microorganism for one host-species does not justify *a priori* conclusions relative to its virulency for any other host-species.

The Site at Which the Parasite Gains Access to the Host.—The importance of this factor for successful inauguration of infection

¹ Jour. Exper. Med., 1924, **39**, 137.

² Ann. de l'Inst. Pasteur, 1924, **38**, 78.

³ Jour. Exper. Med., 1924, **39**, 191.

will become clear by a few concrete instances. Ordinary pyogenic staphylococci and streptococci can cause infection through a break in the continuity of any part of the skin or mucous membranes; while subcutaneous introduction of typhoid bacilli in man will not cause typhoid fever. By way of contrast, viable typhoid bacilli entering the duodenum in sufficient numbers will produce typhoid fever, although in cases of severe oral sepsis we see individuals who have swallowed pyogenic cocci through several years with apparent impunity. Similarly, tetanus bacilli are frequently ingested by the herbivora with no ill effects while these same microorganisms introduced through a wound in the skin will rapidly bring about death. Colon bacilli are almost harmless commensals in the intestinal lumen; in the peritoneum they early bring about a fatal peritonitis.

The importance of the site of entrance is obvious in experimental work. This is clearly indicated by some work of Hartoch and his collaborators.¹ Intraperitoneal injection into guinea-pigs of 0.0002 of an agar slant culture of a paratyphosus B bacillus was fatal; subcutaneous injection of 0.002 of an agar slant culture of the same strain was also fatal, while when the injection was intracutaneous (subepidermal) 0.05 of an agar slant was not only not fatal but did not call forth any serious general reaction.

Dealing with the pyogenic cocci, the intravenous route is most effective. A general infection can in this way be established with a smaller number of bacteria than when other routes are used. Intraperitoneal inoculation is a close second; while subcutaneous introduction is usually a poor third. On the contrary, recent studies on anthrax infection indicate that the skin itself is the most vulnerable portal.² In special instances as in work with meningococci and the virus of rabies of poliomyelitis or of encephalitis, use is made of intracerebral or intracranial injections.

Brown and Pearce³ report that although it is possible to induce syphilitic infection in a rabbit by simple instillation of a suspension of *Treponema pallidum* into the conjunctival sac, still the infection produced in this manner differed from that produced by intracutaneous or testicular inoculation. The course tended to be mild or asymptomatic. The special interest of this observation lies in the bearing it may have on obscure and atypical cases of human syphilis.

¹ Centralbl. f. Bakteriöl., 1922, **93**, 534.

² Besredka: Ann. d l'Inst. Pasteur, 1921, **35**, 421. Plotz: Ibid., 1924, **38**, 169.

³ Jour. Exper. Med., 1924, **39**, 645.

The "Resistance" of the Host.—The field covered by this phrase is very complex and extensive and is considered under the headings of natural defenses against infection, immunity, antibody formation and phagocytosis.

KOCH'S POSTULATES.

The evidence that is classically required to prove that a particular microörganism is the "cause" of an infection is summarized by Koch's postulates:

(1) The microörganism must invariably be present in the infected host; (2) it must be isolated and cultivated artificially for several generations; (3) when then inoculated into a susceptible animal it must reproduce the essential features of the disease; and (4) it must be recovered from the body of such an animal.

The meaning of these postulates can easily be grasped by reference to a concrete case, *e. g.*, anthrax.

1. The anthrax bacillus is invariably present in the tissues and fluids of the infected subject.

2. This microörganism is easily isolated and cultivable for an indeterminate number of generations on artificial media.

3. A minute quantity of such a culture introduced into a susceptible animal, *e. g.*, white mouse, will rapidly induce a condition which is comparable to the condition presented by the man or sheep from which the microörganism was originally isolated.

4. From this white mouse the anthrax bacillus can readily be recovered.

This completes the circle and the fulfilment of these four requirements or postulates is accepted as proving that the anthrax bacillus is the exciting cause of the infection known as anthrax or splenic fever.

Koch's postulates have been satisfied in the case of a large number of the infectious diseases; but other types of evidence are acceptable as creating a strong presumption of the culpability of a particular microörganism. For example, the constant presence of a microörganism in the lesions of an infection, the demonstration in the patient's blood serum of antibodies for the antigens of that microörganism, the prevention of the disease or its amelioration by the use of vaccins or other specific therapy, involving the use of that microörganism; facts of this nature are in general regarded as possessed of almost or quite the same validity as the fulfilment of Koch's postulates.

MECHANICAL DAMAGE BY BACTERIA.

Bacteria inflict during an infection damage of many varied types. Most of this damage is attributable to their chemical composition or to that of their products. Very little is attributable to their acting in a purely mechanical way. It is true that infected fragments of intravascular blood clots (thrombi) may be carried along in the blood stream. The course of such a fragment may be arrested where the lumen of the bloodvessel is too narrow to permit its passage and this arrest may seriously interfere with the nutrition of the parts supplied by the occluded or partly occluded vessel. The microorganisms passively transported in this way may gain a foothold in the tissues whose resistance has been lowered and there may arise consequently a new nidus of infection within the patient. This is a type of embolic dissemination of an infection within the host. We see examples of this following an infective endocarditis, a phlebitis, or puerperal septicemia. Clumps of bacteria themselves, not encased in a blood clot, may occasionally act as emboli. Hepatic or renal abscesses with the pyogenic cocci may follow a blow or other trauma involving a cutaneous infection, such as, *e. g.*, a boil or carbuncle. It is possible that microorganisms, supplied with great invasive powers but with relatively low toxicity and which occasion a bacteriemia, may so multiply within the circulating blood that finally they occlude the finer capillaries and in this way induce death. This has been described of the anthrax bacillus. Such instances as well as all instances in which damage of a mechanical nature is done are, however, relatively few and of small importance.

CHEMICAL NATURE OF BACTERIAL PATHOGENICITY.

Bacteria act upon a variety of organic compounds, *e. g.*, carbohydrates, fats and proteins, and split these up into smaller, less complicated molecules. Some of these products of bacterial activities are more or less poisonous. Harm may result when such products, *e. g.*, ptomains, are contained in the food ingested. Likewise in cases of moist gangrene and the necrotic foci of larger neoplasms, saprophytes gain access to the dead tissues. The resulting disintegration furnishes poisonous substances which are absorbed into the system of the patient. To this condition the term *sapremia* has been applied. Ptomains, also known as "animal alkaloids," are common products of the action of bacterial enzymes on proteins, lecithins, etc. They are soluble basic nitrogenous substances, free

from or poor in oxygen, and of varying degrees of toxicity. Most of them are not very poisonous, while some of the most toxic are the result of the activity of non-pathogenic bacteria.

Among the better known types are:

Putrescin, $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$.

Cadaverin, $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$.

Neurin, $\text{CH}_2 = \text{CH-N}(\text{CH}_3)_3\text{-OH}$.

Muscarin, $\text{CH}(\text{OH})_2\text{-CH}_2\text{-N}(\text{CH}_3)_3\text{-OH}$.

Sepsin, $\text{C}_5\text{-H}_{14}\text{N}_2\text{O}_2$.

The three latter compounds are highly poisonous.

It is widely believed that intestinal fermentation or putrefaction, carried on over long periods, acts harmfully on the patient. It is almost needless to point out that even the most common saprophytes may be involved. In spite of the emphasis which has been layed on this subject, there is little unanimity as to the identity of the poisons at work. Indol of course has been considered. This substance is a product of protein disintegration. It is specifically derived from the heterocyclic amino-acid tryptophane (beta-indol-alpha-amino proprionic acid). This is first deaminated to form beta-indol proprionic acid, which is then subjected to decarboxylation. A CH_2 radical is oxidized to CO_2 and H_2O , leaving skatol. Demethylation of skatol leaves indol, $\text{C}_8\text{H}_7\text{N}$.¹ Indol is a common product of intestinal putrefaction, especially in intestinal obstruction involving the small intestine. Obstruction of the large intestine is less effective unless the stagnation involves the ileum, as it may in the later stages of obstruction. Probably it may also be formed in gangrenous conditions, *viz.*, putrid cancers, putrid placentas, and putrid purulent exudates. The colon bacillus is likely chief agent in the formation of indol in man. Although its toxicity seems to be relatively slight, still the administration of indol to healthy men in quantities of 0.025 to 2 gm. per day causes frontal headache, irritability, insomnia and confusion. Long-continued injection of indol leads to hypertrophy of the adrenal medulla and slight interstitial changes in the kidneys. Indol is excreted as one of its derivatives, indican, in the urine. The urinary indican content is taken as an index of the degree of intestinal putrefaction. Normal urine contains about 12 mg. of indican per day, which amount is so insignificant in proportion to the above mentioned artificial doses that were found necessary to produce symptoms, that the occurrence of noticeable intoxication from indol may well

¹ Underhill: Physiology of the Amino-acids, Yale Univ. Press, 1915, p. 39.

be doubted under ordinary circumstances.¹ Indicanemia may be present in uremia but it is not a toxic factor. The blood normally contains about 0.05 mg. per 100 cc. In uremia it may rise to 0.2 mg. and as much as 2.2 mg. have been found in one case.²

Much blame for obscure disease has been put upon "intestinal putrefaction" and much ignorance has been covered up by that phrase. Probably the weight of Metchnikoff's name had much to do with the mistake of over-emphasis. Alvarez³ in a critical review has pointed out clearly how little real support exists for many beliefs and doctrines of this subject. The syndrome of "intestinal auto-intoxication," headache, depression, restlessness, irritability, anorexia and poor sleep, is not as readily assignable to the absorption of products of bacterial activity as to reflexes arising from simple mechanical distention of the rectum. Apparently the only cases which Alvarez will accept as definitely referable to bacterial activity, are a relatively small group of alimentary intoxications in children. In the severe types, where acute intussusception is simulated, a great abundance of putrefactive organisms is found in the stools. Acute degenerative and fatty changes are found at autopsy in the liver and kidneys. It should also be remembered that bacteria can pass through the intestinal mucosa and that the condition of subinfection which at times results, may be responsible for some of the symptoms of the so-called "intestinal putrefaction" or "auto-intoxication."

Most of the damage done by bacteria in the genuine infections has been ascribed to the action of various substances, such as, exotoxins, endotoxins, bacterial proteins of Vaughn, bacterial proteins of Buchner, leukocidins, and hemolysins. The subject of allergy is so important that its role in bacterial infection is also considered.

EXOTOXINS.

These are soluble products, synthesized during bacterial metabolism, diffusing easily through the cell wall into the surrounding medium. The simple word "toxin" is often used to describe these compounds. The chemical group to which they belong also includes the snake venoms, the specific poison of eel serum, and the vegetable products, ricin, abrin, and crotin. Many, if not all the substances to be described below as hemolysins and leukocidins are closely related chemically. True bacterial toxins are produced in clinically

¹ Wells: *Chemical Pathology*, 4th ed., 1920, p. 581.

² Wells: *Loc. cit.*, p. 537.

³ *Physiol. Rev.*, 1924, 4, 352.

important quantities, by relatively few bacterial species: *e. g.*, *Corynebacterium diphtheriæ*, *Clostridium tetani*, *C. botulinum*, the bacillus of symptomatic anthrax, *Pseudomonas æruginosa*, Shiga's dysentery bacillus, and the organisms of gas gangrene (the Welch bacillus, *Vibrio septique*, *B. œdematiens*). The streptococcus associated with scarlet fever likewise seems to exert its characteristic action by virtue of a body similar to, or identical with, the true toxins. Similar bodies have been described as produced in small quantities by a large number of pathogenic bacteria, (*e. g.*, see the reference to Parker, made below), but they are not in general regarded as contributing essentially to the pathogenicity of the species or strains in question. For example, sterile filtrates of broth cultures of certain strains of *Staphylococcus aureus* were demonstrated by Parker¹ to have a selective poisonous action for the skin of rabbits. Intradermal injection resulted in the formation of a neutralizing substance (antitoxin).

Almost nothing is known of the chemical nature of toxins. Our knowledge is almost limited to the results of their action on certain mammals, supplemented by some information as to their behavior when subjected to certain physical and chemical agencies. A toxin apparently has never chemically been isolated. According to Oppenheimer toxins "are large molecular complexes, probably related to the proteins, corresponding to them in certain properties, but standing even nearer to the equally mysterious enzymes with whose properties they show the most extended analogies both in their reactions and in their activities." Toxins are almost entirely held back by dialyzing membranes (colloidal). Light, oxygen, and oxidizing agencies readily destroy them. They are thermolabile, being destroyed by moist heat at 80° C. or lower, but resisting higher temperatures when dried. They are with the exception of the toxin of *Clostridium botulinum*, innocuous when administered *per os*. For example, 300,000 minimum lethal doses of tetanus toxin can be given by mouth to a guinea-pig without producing any disturbance.² Similarly large doses of tetanus toxin can be given with impunity to mice, although previous administration of bile or bile salts breaks down the protective mechanism, whatever it may be.³ They exert marked and intense activity in very minute quantities, relative to the masses of the substances they attack. As pointed out by Jordan,⁴ the minimal fatal dose of strychnin for an adult man is

¹ Jour. Exper. Med., 1924, 40, 761.

² Ransom: Deutsch. med. Wehnschr., 1898, 24, 117.

³ Dietrich: Klin. Wehnschr., 1922, 1, 1160.

⁴ General Bacteriology, Philadelphia, 7th ed., 1923, p. 117.

30 to 40 mg., while the minimal fatal dose of tetanus toxin for an adult man is less than 0.23 mg. The contrast looms still larger when we recall that toxins in a chemically pure state are unknown but represent only a small fraction of the material available. This means that only a very small fraction of the 0.23 mg. was effective toxin.

There is apparent a definite period of incubation between the administration of the toxin and the occurrence of symptoms. This interval is the shorter the larger the dose, but is never entirely eliminated even when many times the lethal dose is given. For example, in the case of mice which are extremely susceptible, the incubation period may be shortened from thirty-six to twelve hours if we inject 3600 lethal doses but whatever be the dose, the interval cannot be shortened below eight or nine hours. There is evidence that the toxin molecule consists of two parts: A *haptophore* group which effects its union with the substance to be acted upon, and a *toxophore* group upon which depends its poisonous property. This latter or toxophore group is apparently more labile, *i. e.*, more easily altered with consequent loss of toxicity, than is the haptophore group.

A very distinctive feature of all the true toxins or exotoxins is the property they possess of stimulating when introduced into susceptible animals, the production by the tissues of these animals of specific neutralizing substances, to which has been assigned the name *antitoxin*. The toxin of the diphtheria bacillus within the body of a horse, man or other susceptible animal, results in the formation within that animal of a substance which is capable of neutralizing in definite, quantitative proportions the toxin of the diphtheria bacillus, *but no other toxin whatsoever*. The same statement holds for the toxin of the tetanus bacillus, or for that of the bacillus of botulism or for any other toxin, including snake venom, eel serum, ricin, abrin, and croton. The antitoxin which neutralizes and renders innocuous tetanus toxin is completely inert toward any other toxin. The phenomenon of specificity which is so frequently seen in the study of infections is nowhere better exhibited than in the neutralization of a particular toxin by its homologous antitoxin.

Another important phenomenon which assumes prominence in the study of toxins, is that of selectivity. This is very apparent in the case of tetanus toxin. Judged from this stand-point this "substance" is a mixture of two compounds, one of which has a special affinity for the nervous tissue and is in consequence called

tetanospasmin, while the other moiety has a special affinity for the erythrocyte, lysing this cell, and called in consequence *tetanolysin*. Saturation of a solution of tetanus toxin with brain tissue, with subsequent removal of the brain tissue by centrifugalization, will leave a supernatant fluid devoid of tetanospasmin but still containing tetanolysin. Similarly saturation of such a solution with erythrocytes will exhaust the tetanolysin content but leave untouched the tetanospasmin. In the case of diphtheria, the toxin produces necrosis at the site at which the bacilli are growing, and also affects nervous tissue, particularly manifesting this side of its action by motor paralyses of the palate and heart.

HEMOLYSINS.

An action similar to that of the tetanolysin mentioned above is the property of many bacterial species; some pathogenic and some saprophytic. This action results in the diffusion of the hemoglobin from the cell out into the surrounding medium and the eventual disintegration of the erythrocyte itself. It is obvious from the importance of oxygen to vital processes that anything which interferes with the oxygen-carrying power of the blood is harmful to the organism as a whole. It is certain that not all hemolysis resulting from bacterial activity is attributable to substances of the nature of the true exotoxins. Among the bacteria producing hemolytic effects are *Staphylococcus aureus*, a large group of *streptococci* (*alpha* and *beta* types), *B. perfringens* and *vibrio septique* (Weinberg and Nasta), *B. typhosus* and Kruse's dysentery bacillus and some strains of *B. coli*.¹ The hemolysis, for example in the case of *Staphylococcus aureus*, can be demonstrated and titrated *in vitro*. The microorganism is grown in broth, which is later filtered. The sterile filtrate is added in varying proportions to a constant volume of a suspension of erythrocytes, incubated and the results read. The following table is adapted from Kolmer.²

Amount of filtrate, cc.	Rabbit blood 1 per cent suspension, cc.	Physiological salt solution, cc.	Result.
0.01	1	q.s. 2	No hemolysis.
0.02	1	q.s. 2	Slight hemolysis.
0.05	1	q.s. 2	Marked hemolysis.
0.10	1	q.s. 2	Complete hemolysis.

¹ Castellani: Lancet, 1902, i, 440.

² Infection, Immunity and Biological Therapy, Philadelphia, 1915, p. 240.

Weinberg and Nasta¹ studied the role of hemolysins in bacterial infections. They concluded that these substances, far from being inoffensive, contributed to the general intoxication of the host. For example, a given unit of the toxin of *B. perfringens* plus its hemolysin could kill guinea-pigs, 400 gm. in weight. The same unit, freed from its hemolysin, was not fatal to guinea-pigs weighing more than 200 to 250 gm.

LEUKOCIDINS.

Various bacteria possess the property of forming substances which destroy the white blood and allied cells. The danger of this to the macroörganism will become more obvious as we realize the importance of these cells in the defense against invading parasites. Such leukocidins are produced by the pyogenic staphylococci, the *vibrio septique*, the bacillus of symptomatic anthrax and the tubercle bacillus. These substances are usually considered as chemically related to the exotoxins.

Other cells or tissues than those mentioned above may be injured by soluble diffusible products of bacterial growth. The vascular endothelium is subject to deleterious influences ("endotheliotoxins"). The consequent changes are manifested by altered permeability and hemorrhages. The isolated hearts of rabbits which under appropriate conditions can be kept beating for a long time, can be brought to a standstill in a few moments when there are added to the perfusing fluid the toxic ("cardiotoxic") products of typhoid or paratyphoid bacilli, *B. pestis*, *Vibrio cholerae*, *Hemophilus influenzae*, *B. coli*, *B. dysenteriae*, Friedländer's bacillus, *B. pertussis*, *Diplococcus pneumoniae*, or *Neisseriae gonorrhoeae*.²

ENDOTOXINS.

So far we have considered only the deleterious products of intracellular bacterial metabolism which are diffusible out into the surrounding medium. It was early learned that much of the damage done during many infections could not be attributed to such products. It was found that while sterile filtrates of liquids in which certain pathogenic bacteria had been growing were of very

¹ Ann. de l'Inst., Pasteur, 1920, **34**, 690.

² Nukada and Matsuzaki: Jour. Exper. Med., 1924, **40**, 661.

low toxicity, the bacterial cells themselves even when killed were strongly toxic. It was suggested in explanation of this phenomenon that within the bacterial cell there were certain preformed constituents, poisonous for the animals under consideration, which constituents were not diffusible out through the cell wall but which were liberated and became effective only upon the disintegration of that cell. To such constituents the term *endotoxin* has been applied; and endotoxins have been regarded as responsible for much of the picture characteristic of the infections caused by the representatives of the typhoid-colon group, the vibrio of Asiatic cholera, *B. pestis*, the pneumococci and also the other pyogenic cocci. According to the classical view of the mechanism involved, the protective resources of the host by the destruction and disintegration of the invading bacterial cell and by the consequent liberation of endotoxins occasion an at least temporary and at times marked increase in the damage suffered.

Two negative characteristics serve chiefly to separate the endotoxins from the exotoxins. In the first the non-diffusibility of the former from the intact bacterial cell has already been noted. In the second place the endotoxins are incapable of stimulating the production by the invaded animal of specific neutralizing substances, *i. e.*, antiendotoxins. The injection of solutions containing endotoxins results in the production of other types of antibodies (q.v.), *e. g.*, agglutinins, bacteriolytic amboceptors and opsonins. Endotoxins apart from their direct toxicity, appear often to be capable of paralyzing phagocytosis and on this account may be identical with the "aggressins" of Bail.

The concept of endotoxins presented above, *i. e.*, as normal constituents of the living bacterial cell, is that concept which in the history of bacteriology is prominently associated with the name of Pfeiffer. In recent years, however, much doubt has been cast upon the reality of endotoxins in this sense. There is no question but that toxic substances may be derived from bacterial cells after their death, but the question is, did such toxic chemical entities exist *as such* within the living cell or are the toxic products secondary, *postmortem* derivatives? While one may admit that the existence of endotoxins is conceivable, it is not at all certain that the effects which have been ascribed to them are not rather the effects of the products of protein disintegration. This latter suggestion is equally satisfying and is more readily subject to experimental analysis and verification.

BACTERIAL PROTEINS.

The protein content of a bacterial cell is subject to marked quantitative variations. Chemical analysis does not permit as yet of the refinement necessary to distinguish between different bacterial species. Qualitatively we know that proteins are constantly present and that the true nucleoproteins predominate. The presence of globulins and nucleoalbumins has been described. Many of the usual amino-acids have been identified in the products of the hydrolysis of bacteria. On serological grounds though not from the classical methods of chemistry, we know that delicate, but distinctive and constant, differences in the nature of their proteins are demonstrable as characteristic for even the most closely related of bacterial species and strains.

The protein of one species of plant or animal when introduced directly into a different species of animal without having been subjected to the regular digestive processes of the recipient species, will call forth more or less severe disturbances. Bacterial proteins are no exception in this respect. In fact, their toxicity often appears to be higher than that exhibited by proteins from many other sources. The possible role of bacterial proteins in infections was first emphasized by Buchner. This author observed that these proteins introduced into animals, attracted polymorphonuclear neutrophilic leukocytes. This effect is known as positive chemotaxis and probably constitutes an important stimulus in the suppurative inflammatory processes associated with infections. According to this view, consequently, the purulent or suppurative features characteristic of many infections are referable to the action of these bacterial proteins. While the mobilized leukocytes by ingesting the invading bacteria tend to overcome the infection, their unlimited accumulation is not an unmixed blessing. As a sequel of such a process may be cited the peritonitis following a perforating appendicitis.

Besides this pyogenic effect of bacterial proteins attention has more recently been called by Vaughn and his co-workers to another fact which may be of considerable importance in appreciating the ways by which bacteria damage the host. These students found that any protein no matter what be its origin, bacterial or other, could by appropriate methods be split into two moieties, a toxic and a non-toxic product. A similar splitting is assumed to occur in Nature by enzymes elaborated by the cells of the invaded animal. The toxic constituent of the protein molecule is not specific in its

action but is common to all proteins. The differences which are to be seen in the several infections caused by different bacterial species, according to this view, are ascribable not to different toxic substances but rather to the different locations in the body at which these toxic split-protein products are liberated, *i. e.*, according to the distribution of the particular bacterial species concerned. This distribution is largely a function of the invasive properties of the parasite.

The toxic moiety is often extremely poisonous. For example, 10 to 20 mg. of that obtained from the colon bacillus will kill a guinea-pig within ten minutes. Locally at the site of injection of smaller quantities marked inflammation and necrosis result.

Before leaving the subject of the toxicity of proteins, attention should be called to an observation which may eventually contribute to our knowledge of how bacteria damage the host. Suspensions of foreign particles of very minute dimensions apparently are capable of disturbing the colloidal equilibrium of the plasma and other body fluids. Bacterial cells may act as such particles. As a result, the antitryptic property of the plasma is decreased. Under such circumstances blood enzymes may become abnormally operative and split the protein molecules of the blood and tissues. The resulting split-protein products conceivably include toxic compounds which may add materially to the damage done. In this case the immediate source of the poisonous substances is not the bacterial cell but the proteins of the macroorganism itself.

Finally another phase of the relation of proteins to bacterial infection involves the broad subject of allergy—which will be considered in the following section.

ALLERGY.

The condition of unusual or altered reactivity to a (chemical?) stimulus is known as *allergy*. This has long been recognized in the case of drugs, *e. g.*, the idiosyncrasies or increased susceptibility to cocain, morphin, strychnin, atropin, pilocarpin, salicylates or iodids. The reported hypersensitivity to novocain or similar synthetic, local anaesthetics may be an instance of this allergic phenomenon.¹ It is also seen in the case of certain foods. Some individuals exhibit disquieting or even severe reactions to milk or shell-fish, in the form of skin eruptions or gastro-intestinal disturbances. Still more curious instances are afforded by those

¹ Lape: Arch. Dermat. and Syph., 1921, 3, 235.

persons who cannot because of asthmatic attacks, sleep on pillows stuffed with goose-feathers but can on pillows stuffed with duck-feathers, or *vice versa*, or who suffer similar attacks when in close proximity to a horse or a rabbit or even when in a confined space where one of these animals has been. The marked sensitivity to certain pollens as seen in those afflicted with "hay"- or "rose-fever" are further illustrations. The following quotation from the Diary of John Evelyn, under date of June 18, 1670, though possibly not referring to a genuine case of allergy is undoubtedly based on the wide-spread recognition by the laity of allergic phenomena. "Lord Stafford rose from table, in some disorder, because there were roses stuck about the fruit when the dessert was set on the table; such an antipathy, it seems, he had to them as once Lady St. Leger also had, and to that degree that, as Sir Kenelm Digby tells us, laying but a rose upon her cheek when she was asleep, it raised a blister; but Sir Kenelm was a teller of strange things."

It has proved convenient and useful to divide allergic manifestations into two large groups: (1) One anaphylactic, and the other (2) non-anaphylactic or anaphylactoid. The term "anaphylaxis" was coined to describe the condition opposed to "prophylaxis." As the latter word is derived from the Greek, indicating prevention or protection, the term "anaphylaxis" indicates increased sensitivity or susceptibility. In addition to this general meaning, a condition must in the light of present knowledge meet certain further requirements before it can correctly be instanced as an example of genuine anaphylaxis. These requirements are:

1. The substance used for sensitization must be in the dosages employed innocuous to the normal animal.

2. The substance must be a soluble antigenic protein.

3. The substance must gain access to the system by some avenue other than the ordinary one of the gastro-intestinal tract. In other words it must be introduced *parenterally*, *i. e.*, subcutaneously, intraperitoneally or intravenously. Parenteral introduction may conceivably occur by absorption through areas of the gastro-intestinal or respiratory tracts deprived of their epithelium. In this way a state of sensitization or anaphylaxis may be encountered in a patient in whose history there has been no known parenteral introduction of the protein in question. The important thing is that the protein shall have reached the body fluids without having had its antigenic properties modified or destroyed by the digestive juices.

4. An interval of time, analogous to the incubation period of

certain infectious diseases, must elapse after the primary injection before a state of sensitization or anaphylaxis appears.

5. The parenteral introduction of the *same* protein into the sensitized or anaphylactic animal will call forth the symptoms of anaphylactic shock. These symptoms for a given species are practically always the same no matter what protein has been used in the primary and secondary injections. The symptoms vary for different species but not for different proteins. The symptoms vary in intensity according to the degree of sensitization and to the quantity and mode of introduction of the protein employed at the second injection. They usually come on rapidly. The possibility that the observed symptoms are caused by capillary thrombosis or embolism must be excluded.

6. If the animal does not succumb it usually rapidly recovers, apparently little or none the worse for the shock, and becomes refractory to further injections of the same protein, *i. e.*, it becomes immune.

7. The anaphylactic condition may be induced in a normal, animal by introducing into it parenterally some of the serum of a sensitized animal. That is, in true anaphylaxis passive sensitization may be demonstrated. Offspring of sensitized mothers are passively anaphylactic.

Anaphylaxis cannot be induced with equal readiness in all mammalian species. The guinea-pig is highly susceptible; the rabbit and the dog can be sensitized with sufficient ease that they are frequently used in experimental studies. Man is more refractory and the rat for example is so highly refractory that some students of the problem have denied the possibility of producing anaphylaxis in this rodent.

For several reasons the guinea-pig is usually chosen for the demonstration of this type of protein sensitization. We shall follow this custom because the consideration of a concrete case will serve to illustrate and emphasize the meaning and significance of the requirements listed above.

Sterile horse serum is usually the protein selected. It is immaterial whether it has been freshly secured or has come from an old stock. Serum which has been kept in the laboratory for ten years has proved satisfactory. The normal, untreated guinea-pig can tolerate with no untoward symptoms relatively large quantities of horse serum, *e. g.*, 20 cc. Astonishingly small quantities may serve for sensitization, *e. g.*, 0.000001 cc has sufficed.¹ One-tenth to

¹ Rosenau and Anderson: A Study of the Cause of Sudden Death Following the Injection of Horse serum, Hyg. Lab. Bull. No. 29, Treas. Dept. United States, 1906.

0.01 of a cc are often routinely used for the primary injection, which is usually made intraperitoneally. Following this there is a latent or "incubation" period of ten to twelve days at a minimum before the state of anaphylaxis is demonstrable. A second injection of the horse serum during this latent period will fail to call forth any peculiar symptoms. After the expiration of this period, *i. e.*, when the guinea-pig is anaphylactic, a second injection of the same protein will be rapidly followed by a complex or characteristic symptoms. The size of the second injection should not be less than 0.1 cc, while 2 or 3 cc often give more striking results when the route is the intraperitoneal. Larger volumes are necessary when the injection is subcutaneous and much smaller volumes suffice when introduced intravenously.

The train of symptoms begins to appear usually within five to ten minutes, but may occasionally be delayed to thirty or forty-five minutes. Respiratory embarrassment is first evidenced by the guinea-pig scratching at its mouth, coughing and sometimes spasmodic, rapid or irregular breathing. Feces are often passed. The animal becomes restless and agitated. This state is soon followed by one of paresis or complete paralysis. The animal is unable to stand, or if it attempts to stand, it falls upon its side; when taken up it is limp. The hind quarters seem more affected than the fore. Spasmodic, jerky convulsive movements now supervene. At this stage recovery may set in and be apparently complete in an hour or less; or severe convulsions may develop which almost invariably are the forerunner of death. This end, when it occurs, is usually accomplished within one hour after the second injection, and frequently in less than thirty minutes.

Other less obvious symptoms can also be recognized in the animal experiencing anaphylactic shock, *e. g.*, drop in blood-pressure, subnormal temperature, and diminished coagulability of the blood. At autopsy the lungs are typically found to fill the thoracic cavity instead of being collapsed, *i. e.*, they are distended and emphysematous. In the above description horse serum has been the soluble antigenic protein used. It would have been possible to have substituted for it a great variety of similar proteins, including beef, sheep, and human sera and the egg-white of various birds. The point to remember in this connection is that the same protein must be used at the second injection as was used at the first. For example it is impossible to call forth anaphylactic shock in an animal sensitized with horse serum by the injection of beef serum or some other protein.

Anaphylactic shock in the guinea-pig at least, and analogous or identical conditions in the human being, may be aborted at the second injection by the timely injection of atropin or epinephrin. Anaphylactically sensitized animals may also be *desensitized* by the repeated injection of the sensitizing protein, each individual injection being less than the minimum required for the induction of shock. Another mode of desensitization is the very slow injection of the sensitizing protein in very high dilution.

Anaphylactic death in the guinea-pig is readily attributable to respiratory difficulties. The pulmonary distention and emphysema so obvious at autopsy is due to the marked contraction of the smooth musculature of the bronchioles. This tissue in this location in this animal is highly developed. In the rabbit the observed symptomatology is traceable to the spasm of the muscular coat of the pulmonary arterioles. Again in this species the muscular development of the pulmonary arteries is remarkable. In the dog, the distinctive symptoms arise from involvement of the liver and splanchnic circulation; here also one notes that the walls of the hepatic veins differ from those of all other animals in the great development of their musculature. The hepatic and splanchnic congestion, characteristic of anaphylactic shock in the dog is explained as resulting from spasm of the hepatic veins. These facts suggested to Wells¹ the possibility that the characteristic features of acute anaphylactic shock in different species depend merely on difference in the distribution of non-striated muscle in the different species. This statement is not intended to mean that all the changes induced during anaphylactic shock are to be so explained.

The striking sensitivity of smooth musculature to anaphylactic shock has been taken advantage of by Schultz, Dale and Weil to develop a most delicate method of testing for the condition of anaphylaxis: A strip of the virgin uterus of a sensitized guinea-pig is immersed in a physiological solution and connected so as to record its rhythmic contractions on a drum. The addition of a trace of the sensitizing protein to the bath will at once call forth exaggerated contractions. (Fig. 43.)

So far we have limited our attention almost exclusively to the general symptoms of acute anaphylactic shock. Sometimes the intoxication runs a chronic course, as in rabbits where progressive emaciation antedates death by some weeks. Evidences of local or localized sensitivity are not wanting. The most classical one

¹ Physiological Reviews, 1921, 1, 63.

is furnished by what is known as the "Arthus' phenomenon." This is merely the development of an edema and eventual necrosis at the site of the injection of the sensitizing protein in sensitized rabbits. Coulter and Pappenheimer¹ have described glomerular lesions produced in sensitized rabbits by injections of bacterial extracts into the renal artery. The lesion is characterized by the production of cell thrombi and endothelial swelling and proliferation.

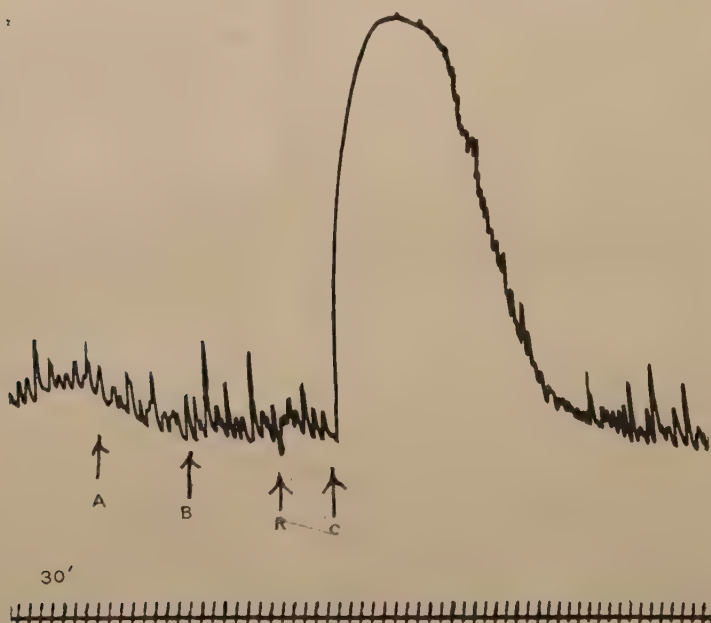


FIG. 43.—Tracing of contractions of guinea-pig uterus. The guinea-pig had been sensitized to horse serum fourteen days previously. At *A* 0.5 cc of sheep serum added; at *B* 0.5 cc of cat serum added; at *R* fluid of both was replaced by fresh Ringer's solution; at *C* 0.1 cc of horse serum added. Note that marked increase in intensity of contractions followed only after *C*. (Dale: *Jour. Pharm. and Exper. Therap.*, 1912, vol. 4.)

Auer² observed a very interesting case which illustrates well how the effects of anaphylactic intoxication may be locally manifested. A sensitized rabbit received the intoxicating injection of the homologous protein. Thirty to forty-five minutes later the ear was gently rubbed with xylol (a hydrocarbon acting not excessively as a chemical irritant in the normal animal). Severe inflammation with the formation of crusts and destruction of tissue often

¹ *Proc. New York Path. Soc.*, 1916, **16**, 80.

² *Jour. Exper. Med.*, 1920, **32**, 427.

followed. Dry gangrene of the entire ear tip may result. The same agent applied in the same dosage and in the same way to the ears of control rabbits causes only a mild inflammation with more or less edema, disappearing in two or three days and leaving a practically normal ear. The ear lesions occurring in the sensitized, reinjected animals are interpreted as a primary anaphylactic reaction, which results from a local autoinoculation of the ear tissues with circulating antigen. The autoinoculation is determined by the inflammation and edema at the site of application of the xylol. The inflamed tissues are more active metabolically than normal tissues and therefore the cells in the inflamed area are affected by more antigen per unit of time than the normal cells. A concentration of antigen, subliminal for the sensitized cells of a non-inflamed area, may thus become effective when the sensitized cells are the site of inflammatory processes. The process described by Auer may theoretically occur in any tissue of a sensitized animal which can show an anaphylactic reaction, *e. g.*, intestines, lungs, heart, skin, nerves, arteries, etc. It is possible that this interplay of conditions may explain a number of functional abnormalities in the human subject.

An interesting instance of local anaphylactic damage is furnished by Opie.¹ Injection of 0.2 cc of horse serum through the thoracic wall into the lung of a sensitized rabbit caused localized consolidation with leukocytes and edema surrounding a central focus of necrosis. The same antigen injected into a normal (non-sensitized) animal is absorbed from the lung without noteworthy change.

The mechanism by which anaphylactic shock or intoxication exerts its damage is unknown. The trend of current thought is to regard the damage as in some way dependent upon a genuine antigen-antibody union. The antigen is, of course, the sensitizing protein. What is peculiar in this reaction is that the antibodies are not free in the blood plasma but are still attached to certain body cells. Consequently the antibody cannot ward off or neutralize any toxic effect of the antigen. The position of the antibody within the cell instead of protecting the cell only serves to render it more sensitive to the action of the antigen or of the antigen-antibody union. According to this concept there is a close relation between anaphylaxis and resistance or immunity. Anaphylaxis may from this stand-point be regarded as a preimmune state. If the stimulus of the antigen at the initial or first few introductions were sufficient to permit the elaboration of antibodies in large quantities with their

¹ Jour. Immunol., 1924, 9, 232.

consequent extrusion into the body fluids, then these free, circulating antibodies would first meet and combine with new influxes of antigen. In this way the antigen would be exhausted before the still intracellular antibodies would be involved, and no harm would be done to the cells. This is the condition of immunity, or at least of humoral immunity. On the contrary, when the free circulating antibodies are relatively few and the fixed, intracellular antibodies are relatively numerous, then influxes of antigen in sufficient quantities will not only exhaust the free antibodies, but will involve the intracellular antibodies or receptors. The result when the quantitative relations are right, is typical acute anaphylactic shock.

The phenomenon of the passive transference of sensitization may be explained in this way. The blood of the actively sensitized animal contains certain free, circulating antibodies in addition to those still attached to the cells. The circulating antibodies introduced into the normal animal are absorbed by the cells of this animal which thereby becomes passively sensitized.

The Bacteriological Aspects of Allergy.—The attention of bacteriologists was first directed seriously to the problems of anaphylaxis and related conditions by the success and wide employment of antitoxic horse serum for the treatment and prevention of diphtheria. They found that guinea-pigs used experimentally and for the standardization of diphtheria antitoxin at times suddenly and inexplicably died. But worse than this, were cases here and there in which the administration of the antitoxic serum to a human being was followed by more or less severe symptoms or even death. Interest in the problem was dramatically focussed by the death under these circumstances of the the two-year old son of Professor Langerhans, who was an international figure in science at that time. The child had had a history of asthma and although not suffering from diphtheria had received as a prophylactic measure 1.2 cc of antitoxic serum. Ten minutes later it was dead.

Of course, the early attempts at an explanation of these unfortunate results looked for it in the antibody content of the serum or in some change in the serum incident upon the treatment of the animal (horse) during its preparation. It was soon clearly shown, however, that normal serum and antitoxic serum were equally efficacious in determining untoward sequelæ.

There is little doubt that genuine anaphylactic shock has been induced in man by the injection of sera into sensitized individuals. Jeanbrau¹ reports a patient who had been given antitetanic serum.

¹ Quoted from Wells: *Loc. cit.*

Three weeks later he received a transfusion of blood from a man who had received a dose of antitetanus serum that very morning. Death of the recipient of the transfusion resulted in a few seconds. Another, less tragic instance is given by Ramirez¹ illustrating passive sensitization in man. The patient who had never had asthma, hay-fever, urticaria or any similar condition, received a transfusion of 600 cc of blood from a man with typical horse asthma, who gave a cutaneous reaction to horse dandruff in 1 to 50,000 dilution. Two weeks later the transfused patient went for a carriage ride and within five minutes had a typical attack of asthma, and a skin test giving a positive reaction to horse dandruff diluted 1 to 20,000 but not to any numerous other proteins.

The fatal result in the case of Professor Langerhans' son and in the case reported by Jeanbrau is very exceptional. There occurs only 1 death ascribable to the antitoxin (serum) for approximately every 75,000 persons injected. These instances are given here merely to illustrate the reality of anaphylaxis or anaphylactoid conditions in man. To minimize the danger, concentrated antitoxic sera, *i. e.*, a high antitoxin content in a relatively small volume of foreign protein have been prepared and careful attention to the patient's history combined with tests to determine his sensitivity to horse proteins is routinely given. Lamson² critically studied the alleged instances of sudden death associated with the injection of foreign substances and came to the following conclusions. "The total number of reported cases of so-called 'fatal anaphylaxis' is much smaller than generally believed. Coincidentally with an enormous increase in the use of foreign serums and other proteins in the treatment of patients, there has been a very marked reduction in the number of fatal cases per unit of population treated . . . A negative skin test may not be an absolute index of the reaction capacity of the patient . . . About 34 per cent of the (fatal) cases give a definite history of asthma or hay-fever. . . . In 8 cases there was a history of a previous injection of foreign protein."

Far more common than the fatal results to which reference has just been made, is the condition known as "serum sickness" which also may follow the administration of therapeutic or prophylactic foreign, *i. e.*, of another species, sera. Usually it is a matter of days before its symptoms present themselves. A local urticaria, or some other form of cutaneous eruption, is first seen at the site of inoculation, later becoming general. The general symptoms include

¹ Quoted from Wells. Loc. cit.

² Jour. Am. Med. Assn., 1924, **82**, 1091.

fever, albuminuria and stiffness of joints. The patient may be very uncomfortable and subjectively depressed. There exists among immunologists considerable difference as to the nature of "serum sickness." Some regard it as a manifestation of genuine anaphylactic sensitivity while others refuse to accept this interpretation.

When an extract of tubercle bacilli (tuberculin) is subcutaneously introduced into a person harboring infection with these organisms, certain symptoms develop which are absent when a non-tubercular individual is similarly treated. Swelling and redness develop at the site of inoculation and the condition of the localized lesions of tuberculosis, such as in the lungs or joints, is aggravated. This latter phenomenon is spoken of as the focal reaction. Possibly as a result of this, some systemic response is noticeable, as a slight rise in temperature and a feeling of lassitude. Parallel results are obtainable under proper conditions when extracts of the cells of other bacterial species are introduced into individuals suffering with the corresponding infections. For example, for the diagnosis of syphilis, Noguchi devised the *luetin* test, in which an extract of *Treponema pallidum* is used; for the diagnosis of typhoid fever, the *typhoidin* test was introduced by Gay, in which the extract of typhoid bacilli is employed, and an extract of the bacillus of glanders, *mallein*, has been long used in the case of glanders. Patients suffering from infections, particularly with a chronic course, certainly exhibit at times a peculiar hypersensitivity to the proteins or the protein-derivatives of the microorganisms causing those infections. When into such patients the corresponding organisms (or extracts derived therefrom) are parenterally introduced, the reactions, local, focal and general, called forth are suggestive of genuine anaphylactic intoxication. However, on account of certain differences their essential identity with this latter condition has not been universally accepted.

Genuine anaphylaxis has nevertheless been observed during infections. Rosenau and Anderson¹ easily induced hypersusceptibility in guinea-pigs to protein extracts obtained from the bacterial cell. The first injection was comparatively harmless but after a lapse of a definite period a profound physiological change has taken place. A second injection then of the same extract is followed by the appearance of symptoms resembling those obtained in the case of horse serum. The bacterial species used included: *B. coli*

¹ Studies in Hypersusceptibility, Hyg. Bull. No. 36, Treas. Dept., United States, 1907.

communis, *B. subtilis*, *B. anthracis*, *B. typhosus*, tubercle bacilli, and yeast. Rosenow¹ reported that guinea-pigs may be sensitized by the parenteral injection of an extract of a hemolytic streptococcus, so that they will react to a subsequent injection of extracts of a pneumococcus and a *Streptococcus mucosus*. Likewise these two last named microörganisms may be used to sensitize a guinea-pig to a hemolytic streptococcus. Coulter and Pappenheimer² report the sensitization of rabbits by injections of bacterial extracts. Genuine anaphylactic sensitization of guinea-pigs to the proteins of the tubercle bacillus (in distinction from the debatable "tuberculin" reactions mentioned above) has been observed by Baldwin,³ by Krause,⁴ by Zinsser and by others. Zinsser and Parker⁵ demonstrated the same phenomenon for the typhoid bacillus; Zinsser and Mallory⁶ found by the very delicate and critical Schultz-Dale-

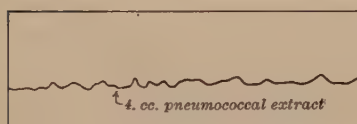


FIG. 44

FIG. 44.—Record of contractions of guinea-pig uterus. The guinea-pig was normal, *i. e.*, had not been sensitized. The contractions were not altered after the addition of the bacterial protein. (Zinsser and Mallory.)



FIG. 45

FIG. 45.—Same, except the guinea-pig had been sensitized. The marked increase in the intensity of the contractions is indicated by the sudden rise of the curve to the right. (Zinsser and Mallory.) Compare Fig. 43.

Weil method the characteristic uterine contractions in the case of guinea-pigs both actively and passively sensitized with pneumococcal extracts. The contractions were comparable in every way to those obtained in experiments with serum or egg-white sensitization. (Figs. 44 and 45). These latter authors note two peculiarities in bacterial anaphylaxis, worthy of remark: (1) Sensitization is experimentally difficult, probably because of the relatively small amount of coagulable protein present in the bacterial body; (2) the margin between the minimum doses which contract the normal and those which contract the sensitized uteri is incomparably smaller than that obtained in analogous experiments with such substances

¹ Jour. Infect. Dis., 1911, **9**, 190.

³ Jour. Med. Res., 1910, p. 119.

⁶ Jour. Exper. Med., 1917, **26**, 411.

² Loc. cit., 1916.

⁴ Am. Rev. Tuberc., 1917, **1**, 65.

⁵ Jour. Immunol., March, 1924.

as horse serum, egg-albumen etc., perhaps because of the unavoidable admixture of primarily toxic substances in bacterial extracts.

In human pathology, the primary and secondary lesions of syphilis are often found containing vast numbers of spirochetes while in the tertiary stage when the disease has settled into a chronic course, although the tissue reaction (the gumma) is greater and more extensive, the spirochetes are demonstrable in relatively far fewer numbers. The reactivity of the tissues in primary and secondary syphilis to the spirochete is far less than is the case in tertiary syphilis. This condition has received an anaphylactic interpretation, whose probability is strengthened by analogy with the observation of Auer described above. This investigator found that the tissue response and involvement to localized injury was greater in rabbits undergoing anaphylactic shock than was the case in the controls. Some types of asthma, as another case from human pathology, have been regarded as instances of bacterial anaphylaxis or hypersensitivity. In such cases, chronic infection is present in the form of a bronchitis. The benefit at times following the administration of autogenous vaccins has been ascribed to a "desensitization." This paragraph can only serve as an illustration to the summary of Zinsser.¹ "There can be little doubt about the fact that both general and localized hypersensitiveness play an important role in infectious disease. Whenever an infection becomes subacute or chronic, the body may become sensitized to the coagulable protein in the bacterial body Thus in all infectious diseases which last any length of time, we can count upon anaphylactic phenomena to participate in the general symptomatological and pathological picture."

DENTAL ASPECTS OF BACTERIAL SENSITIZATION.

Duke² mentions the possibility that certain effects of oral infection may be anaphylactic in nature. Among the symptoms associated with anaphylaxis in the human being are angioneurotic edema, urticaria, erythema multiforme and certain types of eczema. These conditions at times rapidly clear up after the removal of periodontal or other chronic infections.

Price³ discusses at some length the relation of anaphylaxis or sensitization to certain phases of oral infection. Certain disturbances in the dermal tissues, mucous membranes of the nose and throat, lacrimal tissues, mucous membranes of the bronchioles

¹ Textbook of Bacteriology, New York, 5th ed., 1922, p. 372.

² Oral Sepsis, St. Louis, 1918, p. 99.

³ Dental Infections, Oral and Systemic, Cleveland, 1923, 1, 364.

and air passages, as asthma, and the mucous membranes of the digestive tract, are regarded as manifestations of anaphylactic intoxication. These manifestations may entirely and apparently permanently disappear with the removal of the dental infections.

Kolmer¹ has used the allergic skin tests to determine whether or not sensitization exists to bacteria isolated from periapical infections. A positive skin reaction obtained with a microorganism is interpreted as evidence that it is causing some systemic involvement, in which case its incorporation into an autogenous vaccin would seem rational.

The general and focal exacerbations which follow at times the extraction of teeth, the instrumentation of a pyorrheic pockets, or the administration of a vaccin prepared from organisms isolated from the oral infection in the patient treated, may rest in part at least on an anaphylactic condition. The microorganisms growing and succumbing at the site of the oral focus have sensitized the patient. The vaccin or the surgical manipulation of the infected parts have introduced an unusually large quantity of antigen (the bacteria themselves or their products) into the circulation. This occasions the general reaction of fever and malaise and the focal reaction at the sites where the infection has become secondarily localized. For example under such circumstances in an arthritic, the affected joints may become reddened, swollen and tender. This is a phenomenon suggestive of what occurs when tuberculin is injected into a tuberculous individual.

¹ *Infection, Immunity and Biological Therapy*, Philadelphia, 3d ed., 1924, p. 685.

CHAPTER XII.

PROTECTION AND DEFENSE OF THE HOST AGAINST BACTERIAL INVASION. THE NATURAL DEFENSES.

IN the first place the fact should be emphasized that the condition termed "life" *per se* confers marked resistance against infection. A living cell or macroörganism opposes certain hindrances to invasion by microörganisms, which hindrances are not to be seen in the case of dead cells or macroörganisms. The data on which this observation is built, have been available to man for countless ages. No one knows when it was first realized that a living body did not decay while a dead body did decay. As long as the macroörganism is alive the vast majority of all microörganisms are incapable of damaging it. These saprophytes and saprozoa, as we call them, once the macroörganism dies, almost at once invade the cells and tissues and readily accomplish their disintegration. This general invasion sometimes occurs in the agonal period, slightly anticipating death. This event indicates the striking collapse of the defensive forces which occurs when the equilibria which characterize living protoplasm, are seriously threatened or altered.¹ In the early days of experimental embryology it was found that the continued life of the mature ovum (in the absence of parthenogenetic stimuli) depended upon its fertilization by the spermatozoön. Without this event, the ovum soon died and became a prey to swarming bacteria. This phenomenon is not limited to the animal kingdom but is also exhibited by plants, as Bier has pointed out.² This is only a particular instance of the generalization indicated above, forced upon human experience from time immemorial. This difference in the susceptibility to infection between living and dead cells is so obvious that usually it is considered, if it be sensed at all, as without material significance. On the contrary it is a fundamental fact. The factors determining this difference constitute a part of the *sine qua non* of life itself. The identification and evaluation of these fundamental factors have not yet been accomplished.

¹ For a study of terminal infections, see Flexner (Jour. Exper. Med., 1896, 1, 559).

² München. med. Wchnschr., 1924, 71, 491.

There is also a very obvious difference between the organ systems that are derived from ectoblast, entoblast or mesoblast, in respect to their resistance to disease. The development of this thesis we owe to Pearl¹ " . . . in man, . . . , about 57 per cent of all biologically classifiable deaths result from a breakdown and failure further to function of organs arising from the endoderm . . . , while but from 8 per cent to 13 per cent can be regarded as a result of breakdown of organ systems arising from the ectoderm. The remaining 30 to 35 per cent of the mortality results from failure of mesodermic organs." The percentages given include all causes of death, infectious or non-infectious, but it is unlikely that the relative values of these percentages would be materially changed if only deaths from infectious disease were considered. We have only to recall the importance of pulmonary tuberculosis, the pneumonias, and the obscure intestinal affections of infancy, in order to sense the weakness of the derivatives of the endoderm. The factors upon which depends this differential resistance to infection, on the part of the derivatives of the three germ layers, are in part known and in part probably still unsurmised.

There are certain obvious conditions, circumstances and properties which effectively contribute in protecting the living metazoan body against infection. The significance of factors of this nature will be developed in the following paragraphs.

In this category we place the skin and mucous membranes, the hair and nails, the flow of secretions or excretions, and some of secretions themselves, the beating of cilia, the blinking of the eyelids, the peristalsis of the intestines, the symbiosis and antibiosis of the intestinal flora, etc. The senses of taste and smell have some protective value in this connection. The olfactory nerves are very sensitive to methyl mercaptan. As little as one twenty-three millionth mg. in a liter of air can be distinctly recognized. Bunge² suggests a teleological explanation for this. The presence of toxalbumins which although the most poisonous products of putrefaction are non-volatile and therefore without odor, is indicated by the evil smell of methyl mercaptan which is produced along with the toxalbumins. It must be remembered that the senses of taste and smell are not infallible. While it is probably safe, in the absence of more complete knowledge, not to eat or drink what is offensive to these senses, the converse is not invariably

¹ *Biology of Death*, 1922, p. 141.

² *Organic Chemistry for Medical Students*, 1907, p. 39 (translated by Plimmer).

true. For example, water may be perfectly clear and tasteless although it carries an infective dose of typhoid bacilli; one should never be satisfied with milk, merely because it is sweet and rich.

The intact, cornified epithelium of the skin is an efficient barrier to most types of microorganisms. Many bacteria live on and in the folds of the skin, around the hair follicles and in the ducts of glands. Some of these are pathogenic but are being normally constantly removed by the desquamation and by the flushing effect of the sweat and sebaceous excretions. The protective effective of the nails at the tips of the digits is obvious. The eye-lashes are filters for the coarser particles of dust and the hairs of the nostril probably perform much the same function.

The mucous membranes when covered with stratified, squamous epithelium, are only less impenetrable than the skin to bacteria. The moisture, darkness, warmth, abundance of food supply, and thinner epithelial layer account for the difference. The exciting cause of syphilis, *Treponema pallidum*, is usually accredited with the ability of penetrating an intact mucous membrane. Experimental support has been adduced for this view by Brown and Pearce.¹ By simple instillation of a suspension of the spirochete in question into the conjunctival sac or penile sheath of rabbits, they were able to induce syphilitic infection, which tended to run a mild atypical course. In this work it is hard to rule out some mechanical or chemical irritation produced by the vehicle, which would increase the permeability of the mucosa; in natural conditions obtaining in contraction of syphilis by man, it is difficult to see how the presence of minute, even microscopical, abrasions or scratches of the mucosa can be eliminated.

In the oral cavity the protective function of the epithelium is emphasized by the fact that the Achilles' heel is that line surrounding the tooth where the epithelium of the gingival trough ends by abutting against the enamel or cementum. If the views of Gottlieb² and of Orban and Koehler³ relative to the "Epithelansatz," be confirmed, it is obvious that Nature also has been aware of the menace inherent in this relationship.

In the gastro-intestinal segment proper of the alimentary canal, the epithelial defense is much weakened by the fact that it is reduced to a single layer of cubical or columnar epithelium. Its importance, however, under natural conditions is indicated by the following

¹ Jour. Exper. Med., 1924, **39**, 645.

² Deutsch. Monatschr. f. Zahnhlk., 1921, **39**, 142.

³ Ztschr. f. Stomat., 1924, **22**, 355.

observations. It is recorded¹ that Pasteur at first met with ill success when he attempted to prove that the endospores of *B. anthracis*, when given with food were capable of causing anthrax in sheep. The difficulty was simply overcome by giving along with the endospores hay which was full of the prickles of dried thistles. Under these circumstances the infection was readily induced. Cobbett² has observed the blood and internal tissues of normal grouse, rabbits and lambs are approximately sterile. By way of contrast, numerous intestinal bacteria are to be cultivated from the blood and internal tissues of the grouse infested with intestinal worms, of the young rabbit with coccidia, and of lambs



FIG. 46.—Break in superficial epithelium, showing entrance of bacteria into the mucosa. $\times$ O. I. $\frac{1}{2}$, ocular 3. The bacteria are the small, deep black dots and dashes. (Booker: Johns Hopkins Hosp. Repts., 1897.)

with nematodes. The difference is ascribed to the injury suffered by the intestinal mucosa in these cases. (See Fig. 46). Even under normal conditions bacteria succeed in penetrating the intestinal mucosa in small numbers. One of the important functions of the mesenteric lymph nodes and liver is to remove these microorganisms from the circulation before they can be distributed through the body. In case they pass through these organs they still have the gamut of the pulmonary endothelium to run.

There is an important group of workers, beginning with von

¹ Vallery-Radot: Life of Pasteur, 1915, p. 264.

² Causes of Tuberculosis, 1917, p. 152.

Behring and later Calmette and his students, who regard the intestinal tract as the chief portal through which tubercle bacilli enter the body. The ordinary localization in the lungs is looked upon as secondary. Even though most bacteriologists today accept the simple view of an inspiratory origin for pulmonary tuberculosis, probably all would agree that at times the infection may come from tubercle bacilli which had penetrated the intestinal mucosa.

That the epithelium of the intestinal mucosa is not the only factor in warding off bacterial invasion is indicated by the results of the work of Warren and Whipple.¹ They found in the dog that although a suitable dose of roentgen-rays will largely leave the crypts and villi devoid of epithelium and exposed to swarms of bacteria, no overwhelming invasion of the tissues, lymph and blood by intestinal bacteria occurs. Under these conditions there is a profuse excretion throughout the intestinal tract, clinically manifesting itself as vomiting and diarrhea. Fluid escapes through the mucosa and there is emigration of leukocytes. This outflow may be "one of the protective factors which stand between the bacteria of the intestine and the tissues."

Other defensive resources would seem to be also available. Adami² found in sections of healthy human mesenteric lymph nodes, in general, definite bacteria showing morphological signs of degeneration. Killian³ observed that virulent diphtheria bacilli, streptococci and pneumococci could at times pass through the intestinal mucosa of guinea-pigs and mice. In such cases these bacteria, however, could only rarely be recovered in the cervical or mesenteric lymph nodes, in which instances the recovered organisms usually exhibited remarkable alterations. Morphologically they showed signs of degeneration; virulency was depressed or lost and growth on artificial media was very slow.

The mechanical flushing effect of secretions and excretions in their discharge, washing bacteria out of ducts and off surfaces, is a powerful force against infection. Reference has already been made to the elimination of the bacteria from the skin by the flow of sweat and sebaceous secretions. The importance of the secretion of the lacrimal gland is seen in cases where its activity is handicapped, as for example often occurred following operations on the Gasserian ganglion for tic douloureux. Dust and bacteria instead of being washed down the lacrimal canal, determined a conjunctivitis or

¹ Jour. Exper. Med., 1923, **33**, 713.

² Jour. Am. Med. Assn., 1899, **33**, 1511.

³ Ztschr. f. Hyg., 1924, **102**, 262.

keratitis with at times consequent blindness. This danger is much diminished or eliminated by newer methods which are directed to a section of the sensory root alone. The normal flow of saliva effectively prevents ascending infection of the corresponding glands. Where such secretion is interfered with, as has already been noted, glandular invasion by the oral bacteria at times follows, as in some types of postoperative parotitis. The studies of Pickerill and Gies have called attention to the importance of a free, abundant flow of saliva for the prevention of dental caries. Alkalinity of reaction and amylolytic power to dissolve food débris are factors not to be ignored, but probably are of secondary importance in comparison with the mechanical flushing effect of an abundant flow of saliva. The normal flow of the bile and of the urine is usually effective against the institution of ascending infection of the biliary and genito-urinary tracts. The constant outpouring of the secretions, particularly of the small mucous glands, onto the respiratory and gastro-intestinal tracts, not only cleans the walls but also the surfaces of these tracts.

A somewhat analogous mechanical function is to be seen in the peristalsis of the intestine, which normally daily is responsible for the elimination of billions of bacteria. Some of Koch's early work in establishing the pathogenicity of the vibrio of Asiatic cholera is of interest in this connection.¹ Guinea-pigs could not be consistently infected when fed with food carrying this bacterium, unless the acidity of their gastric contents had been neutralized with sodium bicarbonate and the peristalsis inhibited by administration of opium. The abnormal reactions of sneezing, coughing, vomiting, and diarrhea may in part be regarded as mechanisms which are directed to the elimination of infected material. The stroke of the cilia of the respiratory tract and of the Fallopian tube constantly sweeps the bacteria-laden, viscid mucus to the outside.

Access of bacteria to the alveoli of the lungs is hindered, when the air is inspired through the nose. Contributing to this we have the filter of larger hairs just inside the nostrils; the slackening of the air current by the convolutions of the turbinates; the falling of dust and bacteria onto these mucus-covered surfaces where the tenacity of this secretion holds the foreign particles for later removal; the reflexes of sneezing and coughing; the outward sweep of the cilia. In spite of these mechanisms, bacteria and other foreign bodies apparently reach the deeper portions of the lungs with rela-

¹ Deutsch. med. Wchnschr., 1885, Nr. 37 A; reprinted in *Gesammelte Werke von Robert Koch*, 1912, Leipzig, 2, 79.

tive frequency. Some of the literature on this subject was summarized by Beitzke.¹ Rabbits were killed immediately or shortly after the inhalation of *Aspergillus* spores. These spores were recovered in the lungs. In another set of experiments the animals were allowed to inhale air into which tubercle bacilli had been sprayed. Five to ten minutes later they were killed and different parts of their respiratory tract were cut out and injected into guinea-pigs. The results indicated that the bacilli go at least as far as the finest bronchi. Under similar conditions, but in another piece of work the tubercle bacilli were recovered from the alveoli. Calmette and Guérin report that under similar conditions, 2 of 4 guinea-pigs injected with such lungs developed tuberculosis.

Cobbett² found that the lungs of normal animals invariably yielded copious cultures of bacteria, moulds, and streptothrices. A very short exposure of guinea-pigs to air into which *B. prodigiosus* had been sprayed, permitted the recovery of this distinctive organism from the deepest portions of the lungs within five minutes of the commencement of the exposure.

Stillman³ allowed mice to inhale air holding pneumococci and hemolytic streptococci in suspension. Rapid penetration of both microorganisms to the deepest air passages occurred. The pneumococci disappeared in a few hours from the lungs and caused no general infection. On the other hand the hemolytic streptococci maintained themselves and usually caused a general infection.

The disappearance of the pneumococci, reported in Stillman's series, is the fate that is usually encountered by microorganisms which succeed in reaching the deeper pulmonary parts. The destruction is largely if not entirely accomplished by large phagocytic cells.

Bacteriostatic or bactericidal properties have been from time to time assigned to the various normal secretions. Tears, the respiratory intestinal or vaginal mucus, and the saliva are either devoid of such properties or possess them very inconstantly and to a very slight degree. This question as it relates to the saliva has been considered by Valude,⁴ Sanarelli,⁵ Miller⁶ and Clairmont.⁷

The gastric juice is decidedly germicidal. This is largely or entirely attributable to its high acidity. The effect of this property

¹ *Ergebn. d. allg. Path.*, 1910, **14**, 308.

² *Causes of Tuberculosis*, 1917, p. 148.

³ *Jour. Exper. Med.*, 1923, **33**, 117.

⁴ *Compt. rend. du Congr. de la tuberc.*, Paris, 1888, p. 258.

⁵ *Centralbl. f. Bakteriöl.*, 1892, **10**, 817.

⁶ *Dent. Cosmos*, 1903, **45**, 689.

⁷ *Wien. klin. Wchnschr.*, 1906-1907, **19**, 47.

is distinctly seen in the much more favorable prognosis in general in cases of gastric perforation than in cases of intestinal perforation. Achlorhydria is often regarded as favoring the institution of intestinal sepsis. Tetanus toxin is detoxicated by gastric juice while botulinus toxin unfortunately is not.

Although most living pneumococci and a few streptococcal strains are dissolved by the action of bile, most bacterial species are little or not at all affected.¹ According to Ninni² fresh guinea-pig bile, diluted 1 to 7 is actively destructive for strong tetanus toxin.

ANTIBIOSIS.

A potent factor normally very successful in preventing infectious agents gaining a foothold in the body, is the microbial flora of the intestine. Under optimal nutritional conditions harmless saprophytes predominate and exert an antibiotic effect upon intruders which might prove harmful to the host. It is scarcely necessary to point out that no claim of purposeful action is made for the saprophytes. The result observed is an instance of natural selection. Dietary innovations or indiscretions exert harm by suddenly destroying the normal equilibrium and by favoring the development of dangerous bacterial or protozoan species. The popularization of butter-milk or other sour-milk preparations, as desirable items in the diet, is due to Metchnikoff. The goal aimed at is to colonize successfully the intestines with bacteria which not only are harmless in themselves but which also exert distinct antibiotic influences upon other types of bacteria at least potentially harmful to the host.³

BACTERIOPHAGE.

Attention has recently been called by Twort and by d'Herelle to an interesting phenomenon which may play a part in the natural defenses of the intestine against infection. The essential fact is this: When sterile (at least so far as bacteria and larger forms are concerned) filtrates of the stools of convalescents from bacillary dysentery are added to broth cultures of the homologous (or auto-

¹ Posselt: Review, *Ergebn. d. allg. Path.*, 1915, **17**, 719.

² *Ann. d'Ig.*, 1921, vol. **31**.

³ The principle of this method has been applied by Utz (*Dent. Sci. Jour. Australia*, 1922, vol. **2**) in the treatment of oral infections with pure living cultures of *Bacillus bulgaricus*. However at the Operative Clinic of the University of Pennsylvania no benefit was clinically discernible after using for this purpose milk cultures or suspensions of the organisms in physiological salt solution from slants, either of *B. bulgaricus* or of *B. acidophilus*.

genous) dysentery bacillus, there sometimes occurs a destruction (lysis) of these bacilli. A small portion of such a lysed culture transferred to another culture of the same bacterium, actively growing, will bring about the same result, namely lysis. Such transfers may be in cases indefinitely repeated. Similar lysis has been observed in a large number of bacterial species, *e. g.*, the various dysentery strains, *B. coli*, *B. typhosus*, *B. paratyphosus* A and B, *Salmonella*, *B. typhi murium*, *B. proteus*, the diphtheria bacillus, *Staphylococcus albus* and *S. aureus*, *B. pestis*, *B. subtilis*, the vibrio of Asiatic cholera, *Pseudomonas aeruginosa*, etc.

This lytic process has been ascribed by d'Herelle to a living, particulate, ultra-microscopic microbe, to which he has assigned the name *bacteriophage*. We are reminded of the lines:

"a flea
Hath smaller fleas that on him prey;
And these have smaller still to bite 'em;
And so proceed *ad infinitum*."

It is of no consequence for our present purposes whether d'Herelle's views upon the nature of the lytic principle be substantiated or one of the rival hypotheses eventually prevail. The lytic principle or bacteriophage is demonstrable in the intestinal contents of normal man, but its activity is increased in the presence of infection or during convalescence. Several different strains of bacteriophage have been recognized each showing differences in the facility with which they dissolve several bacterial species. d'Herelle goes so far as to state: "The bacteriophage plays a preponderant role in all the phenomena of immunity. It is because of its presence that when exposed to infection an individual remains unscathed, and it is because of its presence that an individual, when sick, recovers."¹ Most students of the subject are not prepared to go to this length.

In the genito-urinary system, the constant downward flow of the urine and its discharge at relatively frequent intervals would tend to wash the bacteria from the ureters, bladder and urethra and prevent thereby ascending infections. The frequency of cystitis and infection of the upper passages in cases of prostatic enlargement, is by many attributed largely to the interference with the freedom of discharge. The acidity of the normal urine is another factor which militates against the proliferation of pathogenic bacteria. In the case of the female genital system the discharge of mucus and

¹ Immunity in Natural Infectious Diseases, Baltimore, 1924, p. 306.

the beat of the cilia of the oviducts carrying it toward the outside renders ascending infections, which might engender even a peritonitis, difficult. The reaction of the vaginal secretions is normally acid, a fact whose advantages have already been mentioned in connection with the sweat, gastric juice and urine. The vagina is normally inhabited by relatively characteristic saprophytes and saprozoa which often prevent pathogens from gaining a foothold. It has been suggested that a function of the hymen, still a puzzle to the comparative anatomist and anthropologist, was to protect against the ingress of the ova and larvæ of parasitic arthropods and worms which else might readily gain access while the child was sitting or squatting in the dirt.

The body juices, *e. g.*, blood plasma, lymph, aqueous and vitreous humors, spinal, synovial, pleural, pericardial and peritoneal fluids normally possess certain powers of inhibiting or destroying bacteria or other parasites. These powers are often materially enhanced during an infection or following certain artificial measures. These powers are ascribed to certain constituents of these juices, chemical entities, to which such terms as the following are conventionally assigned: Antitoxins, agglutinins, precipitins, amboceptors or sensitizers, complement or alexin, bacteriotropins, opsonins, etc. The nature and properties of these hypothetical substances will be later considered at some length.

As the last item in this catalogue of the natural defenses, we wish to refer to the ability, widely possessed by many cells, particularly those of mesenchymal origin, to ingest and digest foreign particles. The term "foreign particles," of course, includes bacteria and other parasites. This type of defense is known as *phagocytosis* and because of its importance will be developed further on at some length. Under natural conditions many bacteria which as we have seen penetrate to the deeper structures of the lungs, or which pass through the intestinal mucosa to the portal circulation, are disposed of in this way.

CHAPTER XIII.

PROTECTION AND DEFENSE OF THE HOST AGAINST BACTERIAL INVASION. THE HUMORAL DEFENSE.

IN the actual presence of infection there occurs an increase of the normal defensive resources resident in the body fluids and in the body cells. Historically the study of the defenses in the body fluids has to a large extent been carried out independently of the study of the cellular defenses. For convenience it is still customary to consider the humoral and the cellular defenses separately. It must be remembered, however, that this separation is artificial and that under natural conditions the economy of the macroörganism as a whole does not hesitate to mobilize and utilize all its defenses. It is also necessary to point out, although it will at once strike one as self-evident, that the defenses resident in the body fluids, *i. e.*, the humoral defenses, must have had their origin in cellular activity.

The defenses against infection act: (1) By preventing the multiplication of the parasite; (2) by destroying the parasite; (3) by neutralizing or in other ways rendering innocuous the harmful products of the activity of the parasite.

Defense by interference with the free multiplication of the parasite has been less considered than the other types of defense. Attention has been called to it by Taliaferro¹ in the case of *Trypanosoma lewisi* infection in the rat. The method employed in studying this question "is based on the obvious and well-known fact that a sample taken on one hand from a population undergoing rapid reproduction, with the constant production of young forms and intermediate growth stages, will exhibit much greater variability in size than a sample taken on the other hand from a population in which there is little or no reproduction and in which all of the organisms are full grown adults." The variability is expressed as a "coefficient of variation" which has a higher value the greater the variability. When trypanosomes first appear in the blood, the coefficient of variation is about 30 per cent; a fact indicating a population reproducing at a maximum rate. On succeeding days this coefficient gradually drops until it reaches a plateau at about

¹ Jour. Exper. Med., 1924, 39, 171.

3 per cent, indicating the normal variation of non-reproducing adult forms. Confirmatory of this evidence of an inhibition of reproduction in the parasite, is the observation that in direct examinations of stained blood smears dividing stages are extremely common on the first day after trypanosomes appear in the blood, but gradually decrease until there are none at all during the latter part of the infection. This retardation and eventual inhibition of reproduction are ascribed to the formation of a reaction product present in the blood plasma. This process is distinct from any trypanosomatolytic or trypanosomatocidal phenomenon because while the coefficient of variation is falling, *i. e.*, while the retardation of reproduction is becoming greater, the actual number of

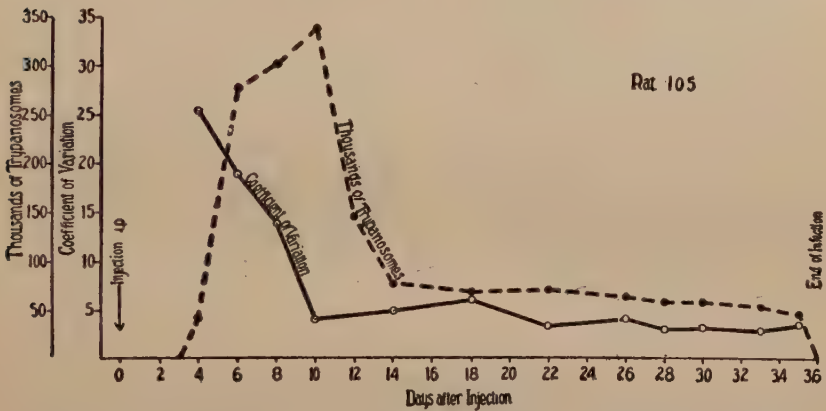


FIG. 47.—Graph showing the comparative measure of the rate of reproduction and the number of trypanosomes per cubic millimeter of blood throughout the course of the infection with *Trypanosoma lewisi*. (Taliaferro.)

parasites in a unit volume of the blood almost invariably increases. (Fig. 47.) The actual destruction of the trypanosomes begins later as a second phase in the host's defense.

Invading, pathogenic parasites may be killed by means of certain constituents of the blood plasma or other humors and by means of the phagocytic (*q. v.*) activity of certain cells, almost or quite all of mesenchymal origin. This parasiticidal activity is normally available against a wide variety of microorganisms; but it is usually capable of great specific enhancement toward a particular invading microbe. To be definite, the blood serum of most normal individuals will at high concentrations rapidly destroy not too large numbers of *Staphylococcus aureus*; in an individual suffering from a

not overwhelming infection with this organism an equal bactericidal effect of the serum will be demonstrable at relatively very low concentrations.

BACTERIOLYSIS.

This property of the serum and other body juices was first extensively studied by Pfeiffer. If cholera vibrios be introduced into the peritoneal cavity of a guinea-pig which has recovered from an infection with that parasite, the guinea-pig remains free from a

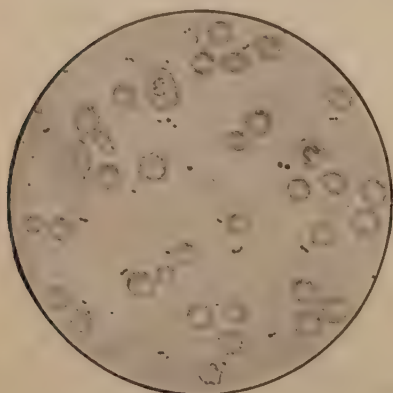


FIG. 48

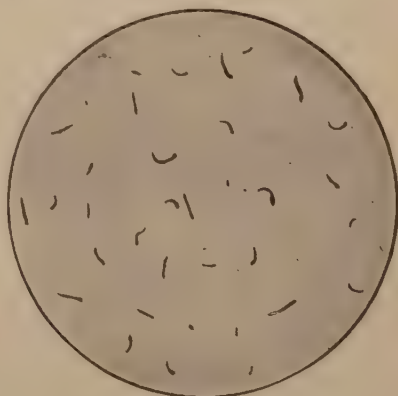


FIG. 49

FIG. 48.—Culture of cholera undergoing bacteriolysis. A positive Pfeiffer reaction. A hanging drop of peritoneal exudate removed from a guinea-pig one-half hour after injection with 1 cc of the suspension shown in Fig. 49 with 1 cc of 1 to 1000 dilution of cholera immune serum. Note that the bacilli are now quite short and coccoid in shape. At the end of seventy minutes the exudate was sterile. What appears to be two or three coccoid forms in apposition is really one bacillus undergoing lysis. (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

FIG. 49.—Culture of cholera before bacteriolysis. $\times 720$. A hanging drop of cholera in normal salt solution prepared from a twenty-four-hour culture of cholera on agar-agar. (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

second disease attack. Microscopical and cultural examination of the peritoneal exudate explains this result. The microorganisms first lose their motility, become swollen, granular, and eventually dissolve away. The disintegration of a parasite under these conditions is known as Pfeiffer's phenomenon (Figs. 48 and 49), and it is demonstrable even *in vitro*.

Pfeiffer further found that if a host were resistant to infection with one kind of bacterium (*A*) but not with a second kind (*B*), only the first kind of bacterium (*A*) was subject to this lytic process,

while the second kind (*B*) was not affected when injected intraperitoneally into the same guinea-pig. This means that Pfeiffer's phenomenon is *specific*. To give a concrete instance, if a suspension of *Vibrio cholerae* be injected intraperitoneally into a guinea-pig which has recovered from infection with that microorganism, lysis readily occurs. If, however, into the same or similar guinea-pig a culture of meningococci be introduced, the lytic process will be far less marked or entirely absent.

Pfeiffer made another significant discovery. The protection afforded by Pfeiffer's phenomenon to the guinea-pig which has recovered from the infection in question, can be transferred artificially to a normal, otherwise susceptible guinea-pig. If some of the blood serum of the first animal be added to the suspension of the bacteria in question and this mixture be injected intraperitoneally into the normal, susceptible animal, lysis will occur and this animal will not become infected.

Finally it was determined that the serum of an immune, resistant animal lost its power of specific bacteriolysis after being heated to 50° or 60° C., when the test was made *in vitro*, i. e., outside the animal body. When, however, such a serum, initially lytic but inactivated by heat, was injected with the corresponding bacterium into the peritoneal cavity of a normal, susceptible guinea-pig, bacteriolysis proceeded as actively as if the serum had never been heated.

The data presented in the few above paragraphs were confirmed by Bordet and an interpretation was offered which is essentially valid today. Bacteriolysis is regarded as being dependent upon the coöperation of two distinct constituents or properties of the blood serum or other body humors. We are not really sure that these two factors are distinct chemical entities. They may rather be regarded as "properties" of one or another of the constituents of these fluids. At any rate for purposes of convenience these properties are customarily "personified" as definite chemical entities. This treatment has advantages when one is first trying to grasp the subject, but throughout all that follows one should remember that no protective substance has been chemically isolated from such immune sera or humors. As little is known of the chemistry of these hypothetical bodies as is known of the toxins, hemolysins, leukocidins or endotoxins mentioned elsewhere. When we speak of the peculiar bacteriolytic factors as distinct chemical entities, it should be with the mental reservation that we may be assuming too much. It is technically permissible and even pragmatically desirable to

make such formal assumptions. The postulates of mathematics and physics belong to this category and have proved their worth. But we should not accept logical abstraction, however useful, as necessarily a reality.

The experimental data furnished by the important investigations of Pfeiffer confirmed and extended by Bordet and many others, suggest the interpretation that bacteriolysis requires the combined action of two distinct factors. One of these is (a) specific, *i. e.*, operative almost or quite exclusively against a particular kind of bacterium; (b) thermostable, *i. e.*, not destroyed or rendered inactive by heating to 50° or 60° C; (c) capable of being temporarily enormously increased under proper conditions. Among such "proper conditions" would be experiencing the infection or undergoing artificially a course of injections with killed suspensions of the parasite in question. To this factor has been applied various terms. A very satisfactory one is *substance sensibilisatrice* suggested by Bordet, although the term probably in widest use in this country is "amboceptor."

The other factor is described best by the negatives to the characteristics of the amboceptor. It is *not* specific. It is relatively thermolabile, being inactivated by temperatures of 50° to 60° C. And it is not increased in quantity by spontaneous infection or by artificial immunization. To this factor the terms *alexin* or *complement* have been applied. Another important difference between amboceptor and complement is that bacterial or other cells are capable of absorbing and removing corresponding, specific amboceptor from serum, but without the presence of specific amboceptor they are unable to attach complement to themselves. For example, cholera vibrios added in sufficient quantity will remove amboceptor from heated (inactivated), anticholera serum. Such vibrios can then be centrifugalized and washed free of this serum, and then when subjected to fresh normal, non-immune serum, they undergo lysis. On the contrary, cholera vibrios first subjected to fresh, normal serum (complement alone), washed free of this and then subjected to inactivated anticholera serum (amboceptor alone) will not undergo lysis.

The actual dissolution of the bacterium or other cell is according to current thought ascribed to complement or alexin. The amboceptor or *substance sensibilisatrice* is regarded as a means of effecting a union between the bacterium and complement or of "sensitizing" the bacterium in some way so that it becomes susceptible to the lytic action of complement. More attention will be given to this later in presenting Ehrlich's side-chain theory.

The reaction of amboceptor and complement and the bacterial or other type of cell destroyed by their combined action is a quantitative one. For the completion of the reaction the relative proportions of these three reagents must be just right. The following table, adapted from Kolmer (1924, p. 408), illustrates this. A word of explanation is called for, and we must anticipate a bit. In discussing the specific lytic effect of a serum it has been found possible to substitute the red blood cell for the bacterial cell. What holds good for the erythrocyte in this respect also proves valid for the bacterium.

Tube.	Amount of inactivated immune serum (amboceptor), cc.	Amount of complement (1 to 20 dilution), cc.	Amount of erythrocytes (2.5 per cent), cc.	Physiological salt solution, cc.	Result after 1 hr. at 37° C.
1	0.1 (0.0001 cc undiluted)	1	1	q.s. 3	No hemolysis.
2	0.15 (0.00015 cc undiluted)	1	1	q.s. 3	Beginning hemolysis.
3	0.2 (0.0002 cc undiluted)	1	1	q.s. 3	Partial hemolysis.
4	0.25 (0.00025 cc undiluted)	1	1	q.s. 3	Just complete hemolysis.

In this instance the quantitative relations necessary for hemolysis were 0.00025 cc of amboceptor-containing serum; 0.05 cc of complement-containing serum, and 1 cc of a 2.5 per cent suspension of erythrocytes. If less amboceptor or complement, or more erythrocytes had been used, hemolysis would not have been complete. One peculiarity, however, must be remarked which distinguishes these quantitative relationships from those which obtain in elementary inorganic chemistry. Within certain narrow limits, the proportions of amboceptor and complement may be inversely varied without interfering with the eventual completion of hemolysis. This means an increase in amboceptor will permit a decrease in complement, or an increase in complement will permit, though to a lesser degree, a decrease in amboceptor without interfering with the eventual completion of hemolysis. This phenomenon is illustrated in the following table:

Erythrocytes, 5 per cent suspension, cc.	Amount of amboceptor.		Amount of complement.	
	Absolute.	Relative.	Absolute.	Relative.
1	0.05	1	0.0080	5.71
1	0.20	4	0.0025	1.78
1	0.40	8	0.0014	1.00

The quantities of amboceptor and complement in each of the three cases were just enough to bring about complete hemolysis. It is obvious that as the quantity of amboceptor increases the quantity of complement needed decreases. All of the specific reactions participating in the defense against infection are of a quantitative nature. They cannot adequately be understood if we neglect this phase.

PFEIFFER'S PHENOMENON.

The significance of the concepts "amboceptor" and "complement" will become clear if we apply them to the case originally described by Pfeiffer. The peritoneal exudate of a normal, susceptible guinea-pig has little or no lytic action on the vibrios of Asiatic cholera. The lytic powers (against this parasite) of the blood-serum and peritoneal exudate can be enormously increased by subjecting the guinea-pig to a preliminary course of injections, first with killed suspensions of this vibrio and then with small doses of the living microorganisms. Such increase as is noted applies only to the particular microbic species which has been used in the immunizing injections. Cholera vibrios (but in this case no other bacterium), introduced into the peritoneal cavity of such an immunized guinea-pig rapidly undergo dissolution. The dissolution can likewise be seen to occur outside of the body under the microscope if *fresh* blood serum or peritoneal exudate of an immunized animal is used.

If such blood serum be allowed to stand for a time or if it be heated to about 56° C., it will lose its ability to dissolve the cholera vibrio. The result will be as if the serum of a normal susceptible animal had been used. However, as the next step will show, such an inactivated serum is very different from the normal serum. If we take a mixture of fresh, unheated, normal serum and of a suspension of cholera vibrios, in which mixture lysis is not occurring, and if to this we add a small portion of the heated (inactivated), non-dissolving, serum of an animal immunized against the micro-organism in question, then lysis will be started and will progress at a high rate.

In terms of amboceptor and complement these events would be described as follows: The immunizing injections increase the quantity of amboceptor in the serum. This is specific because the increased lysis occurs only in the case of the microorganism used for the immunization. The loss of lytic power on heating is due to the

destruction of the other factor, complement. The thermostability of amboceptor is shown by the restoration of lytic power to the heated immune serum, by the addition of fresh normal serum, *i. e.*, solely by the addition of fresh complement. The fact that either fresh normal serum or fresh immune serum are both equally efficacious in restoring lytic power, indicates that complement is not specific and that it is not quantitatively increased by the process of immunization. The lack of specificity of complement is further shown by the fact that fresh normal serum can reactivate, *i. e.*, restore the lytic power to an indefinite number of inactivated (heated) sera containing each a specific amboceptor against a different microorganism. Another noteworthy characteristic of complement is that it may be furnished by an animal belonging to a different species than that which furnished the specific amboceptor-containing serum. For example, inactivated goat or guinea-pig serum, containing amboceptor toward the cholera vibrio, can be reactivated by the addition of fresh, normal serum of the guinea-pig, rabbit, man, goat, rat, etc.

Inasmuch as immunization greatly increases specific powers of bacteriolysis and inasmuch as in this process of immunization the quantity of complement is not increased, it necessarily follows that the increased lytic power is ascribable to an increase in the amboceptor content of the serum.

Pfeiffer's phenomenon appears to its best advantage in the case of the cholera vibrio and a few related forms, *e. g.*, the vibrio of Metchnikoff and that of Finkler and Prior, which latter forms, however, are not pathogenic for man. The spirochete of recurrent fever, the meningococcus and attenuated bacilli of bubonic plague are also susceptible to bacteriolysis. The typhoid and paratyphoid bacilli and *Pseudomonas aeruginosa* are affected but to a lesser degree. Other microorganisms, as the streptococcus, staphylococcus, pneumococcus, the diphtheria bacillus, the anthrax bacillus and pathogenic streptothrices, are according to Bordet (p. 313) not subject to lysis in immune sera. Whether or not this generalization be always valid, immune sera can exert a bactericidal action on at least some of these species.

There is evidence that bacteria which are not susceptible to lysis, such as those mentioned above, may nevertheless be so affected by specific amboceptor and complement that they more readily undergo phagocytosis (see *infra*). It certainly is premature to conclude that amboceptor and complement together are identical with opsonin.

CYTOLYSIS.

It is very interesting from the stand-point of general pathology and more broadly of general biology that bacteriolysis is only a particular case of a general phenomenon, known as cytolysis. The body fluids of at least birds and mammals either possess or may by appropriate treatment acquire the power of dissolving various types of cells belonging to other species, or to the same species in some instances. The appropriate treatment, as in the case of producing bacteriolytic sera, consists in the injection, subcutaneous, intraperitoneal, or intravenous, of the cells of one species into another species. In this way sera may be produced which are capable of dissolving erythrocytes, leukocytes, spermatozoa, ciliated epithelium, the stratified squamous epithelium of the skin,¹ nerve cells, and the parenchyma of liver, pancreas, stomach, thymus, adrenal, and corpus luteum. Theoretically it is possible and it actually has been claimed at one time or another, that specific cytotoxic or cytolytic sera have been produced, effective against almost every type of cell or tissue in the body. Some of the early successes in the production of specific cytolytic sera gave hope that similar sera could be produced against malignant neoplasms. Such hopes have, however, not been realized.

The mechanism of cytolysis is that of bacteriolysis, namely lysis is accomplished by the coöperation of amboceptor and complement. In fact, much that we know of the action of amboceptor and complement is the result of studies in which red blood cells were used instead of bacteria. Bordet discovered that the injection of the erythrocytes of one species (*A*) into a second species (*B*) resulted in the acquirement by the serum of the second species (*B*) of marked powers of dissolving *A*'s erythrocytes, *i. e.*, erythrocytolytic amboceptor or, as it is more frequently called, hemolytic amboceptor was produced in large quantities. This amboceptor is specific, *i. e.*, such serum of species *B* will mediate only in the lysis of *A*'s erythrocytes but not in the lysis of the erythrocytes of any other species, *e. g.*, *X*, *Y* or *Z*.

Bordet's work is of great significance. It helped us to realize that the phenomena of pathology are only particular cases of the phenomena of biology. The conscious assumption of this attitude or view-point is perhaps what is most distinctive and essential in the development of modern medicine. Besides this philosophic consideration, the demonstration of hemolysins afforded a method

¹ Ledingham: Brit. Jour. Exper. Med., 1924, 5, 346.

of great technical value. Certain difficulties stand in the way of establishing and of accurately measuring the lysis of such small colorless bodies as bacteria. In the case of hemolysis, however, simple naked-eye inspection of a test-tube in which the reagents are mixed, will immediately inform us whether lysis has occurred and will also permit a satisfactory estimate of the degree of lysis. In case no lysis occurs in a tube (containing erythrocytes, their corresponding amboceptor and complement, in proper proportions) the erythrocytes remaining intact will settle by gravity to the bottom of the tube and the supernatant fluid will remain colorless. In case complete lysis occurs, there will be no sediment of erythrocytes and the supernatant fluid will be intensely tinted with the hemoglobin which has been freed from the erythrocytes and is distributed in homogeneous solution throughout the fluid. Between the absence of hemolysis and complete hemolysis, all conceivable gradations may occur. Where it is marked, the sediment of erythrocytes will be scant and the tint of the supernatant fluid will be deep; where it is slight, the sediment will be great and the tint of the supernatant fluid will be faint.

By means of this method of studying amboceptor and complement in the case of hemolysis, knowledge regarding cytolysis was advanced rapidly. Without this method, if all research had been exclusively dependent upon studies employing bacterial cells or even other types of colorless cells, progress would have been much more difficult and slower. It has been found that what holds for the cytolysis of erythrocytes also holds for the cytolysis of bacteria (bacteriolysis).

COMPLEMENT FIXATION.

The specificity of amboceptor naturally suggested the possibility of its application to the identification of microorganisms and the diagnosis of infection. Pfeiffer's phenomenon was early used to differentiate the cholera vibrio from closely allied, but not pathogenic, forms. A guinea-pig which had been injected with a known culture of the cholera vibrio and which therefore specifically had the power of lysing this parasite, received intraperitoneal injections of the unidentified but suspected organism isolated from the stools or other source. If lysis occurred the organism in question was identified as a genuine cholera vibrio; if no lysis occurred, the possibility of that identification was eliminated. The true meningococcus is distinguishable in the same way from the parameningococci.

In the above instances the kind of amboceptor was the known

while the isolated organism was the unknown factor. The reverse of this is also useful in diagnosis, namely where the microörganism is the known factor and one is attempting with its aid to determine if the corresponding amboceptor be present in the patient's serum. If such an amboceptor be found, that microörganism is regarded as being a factor in the patient's condition. The procedure almost universally adopted for this purpose is known as the *complement-fixation test*. It should be borne in mind throughout that the test is devised to determine if an amboceptor against a particular, known type of microörganism be demonstrable in the blood serum or other body humor of the patient. It is further necessary to recall that the lysis of a cell is a quantitative reaction, requiring for its completion certain definite relations between (a) the cells, (b) the inactivated, homologous amboceptor contained in the lytic serum, and (c) the complement in the fresh normal serum. The reagents in correct proportions constitute the cytolytic system.

The complement-fixation test may be briefly described as an attempt to complete two cytolytic systems with only enough complement for one. One of these two systems is invariably a hemolytic one, and the other cytolytic system is a bacteriolytic one.

The erythrocytes of the hemolytic serum usually are supplied by the sheep. The corresponding amboceptor is usually secured by injecting sheep's erythrocytes into a rabbit. This furnishes an antisheep-rabbit serum. This is inactivated, *i. e.*, its complement is destroyed, by heating at 56° C.

The bacteriolytic system contains as the cells, a suspension or extract of the bacteria suspected of having something to do with the patient's condition. It is, of course, unknown whether the patient's blood serum or spinal fluid contains a specific amboceptor for the species of bacteria used. The point of the whole test is to determine the presence or absence of such amboceptor. The patient's serum is inactivated by heat to destroy such complement as it contains. The complement that is used in complement-fixation tests is generally furnished by the fresh normal serum of a guinea-pig.

If one set up two cytolytic systems, a hemolytic and a bacteriolytic, this could be represented as follows.

Hemolytic:

- (a) Erythrocytes.
- (b) Inactivated serum with homologous amboceptor.
- (c) Complement (fresh, normal serum of guinea-pig).

Bacteriolytic:

- (a) Suspension of bacteria of Species A,

(b) Inactivated serum with specific amboceptor against *A*.

(c) Complement (fresh, normal serum of guinea-pig).

If the quantitative relations of the three constituents of each system be correct, complete lysis of the cells (erythrocytes and bacteria, respectively) will occur.

In actual complement-fixation work one proceeds as follows: The correct quantitative relations of the reagents are established by preliminary titrations. Into one test-tube are put:

(a) The bacterial suspension.

(b) The inactivated serum of the patient (one does not yet know whether or not this contains the amboceptor needed for cytolysis).

(c) The fresh, normal serum of a guinea-pig (complement).

This mixture is incubated. If the patient's serum contains the amboceptor corresponding to the species of bacterium used, *i. e.*, if the patient is suffering from an infection with that bacterium, then the three constituents of a complete bacteriolytic system will be present. They will react so that all or some of the complement will be used up and will no longer be available for another cytolytic system. In other words complement will be fixed. The resulting bacteriolysis or bactericidal effect, however, is not self-obvious. The subsequent steps really serve only as an indicator, to tell in the first place if the quantity of free complement originally present in the above mixture has been diminished, and in the second place by inference, if the patient's serum contained the amboceptor corresponding to the known species of bacterium used.

The function of the indicator is performed by the addition to the above incubated mixture (bacterial suspension, inactivated serum of patient and complement) of the first two components of a hemolytic system, namely, sheep's erythrocytes and inactivated, antishoop-rabbit serum. If complete hemolysis occur after incubation, this means that the original quantity of complement had not been diminished; this means that the bacteriolytic system was not completed and the only possible missing component was the amboceptor in the patient's serum. This is often interpreted as meaning the absence of infection by the bacterial species used in the test.

If on the other hand, hemolysis be incomplete or absent, this means that the original quantity of complement had been diminished or exhausted (fixed); this means that the bacteriolytic system was completed; this could only occur if the patient's serum contained a specific amboceptor against the bacterium used. Therefore the patient has suffered or is suffering from an infection with that bacterium.

In brief, in complement-fixation tests, complete hemolysis indicates the absence of infection with the microorganism used, that is, a negative result; while incomplete or absent hemolysis indicates infection, of the present or recent past, that is, a positive result. Kendall. (See Plate V).

We have presented here only the principles involved in complement-fixation work and a mere outline sketch of its technic. The actual performance of the test requires a very complicated system of preliminary titrations and controls, much patience, accuracy, and experience and skill in reading and interpreting the results.

Complement-fixation tests may be relied on for the diagnosis of many infections, *e. g.*, gonococcal, typhoid, paratyphoid, glanders, tuberculosis, leprosy, whooping cough, and echinococcal. The Wassermann test or reaction which is so valuable in the diagnosis of syphilis, was originally conceived as a complement-fixation test. This view, however, is known today to have been a mistaken one. The nature of the reaction is not yet known, except that it is not a genuine complement-fixation test. Further reference to the Wassermann reaction will be made in the discussion of syphilis in Part III. In passing, it should be pointed out that syphilis can be diagnosed by a genuine complement-fixation test in which a suspension or extract of a pure culture of *Treponema pallidum* is used. But this test is less delicate and reliable than the Wassermann reaction.

AGGLUTININS.

It was noticed soon after the study of the effects of bacterial infection on the patient's serum was begun, that in some instances when homogeneous suspensions of the responsible bacteria were mixed with the patient's serum the bacteria, if motile, lost their motility and at all events instead of remaining homogeneously dispersed gathered together into clumps. The clumping of bacteria under these circumstances is known technically as agglutination (Fig. 50). The hypothetical substance in the serum responsible for this phenomenon is known as the agglutinin, and the substance in the bacterial cell which initially was responsible for the production of the agglutinin and which by combination with the agglutinin determines the clumping is known as the agglutino-gen.

As in all immunity reactions the phenomenon of specificity is prominent, although not absolute. A given type of cell, *e. g.*, the dysentery bacillus of Shiga, will in the case of a spontaneous infection or under experimental conditons, call forth a great increase

of agglutinin production. This agglutinin will be most strongly operative against the Shiga bacillus, but this particular serum will also prove to have agglutinating power, appreciably above normal, for other types of closely related bacteria. In other words, here as elsewhere specificity is not absolute but relative. This phenomenon of relativity is known as the phenomenon of group agglutinins, to which reference will be made later.

The fundamental mechanism of agglutination is unknown. This much, however, is known: The agglutigen is either a protein or some substance which has so far proved to be inseparably associated with proteins. The corresponding agglutinin has never been chemically isolated and is only known by some of its physical

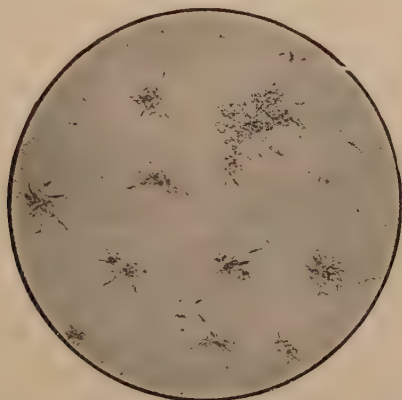


FIG. 50.—A positive agglutination (Widal) reaction in typhoid fever. $\times 430$. Serum from a patient ill about twenty-two days; a 1 to 100 dilution at the end of one hour. (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

properties and by its peculiar affinity for the agglutigen which in a way was responsible for its production. The present tendency is to regard agglutination, along with the other immunity reactions, as essentially colloidal in nature. It has been demonstrated that salts, *i. e.*, crystalloids and electrolytes, are necessary for the clumping. The equilibrium which permits the homogeneous dispersion of the carrier of the agglutigen, is disturbed under the influence of the agglutinin. This disturbance permits the "precipitation" of the agglutigen-agglutinin complex by the electrolytes of the serum. Michaelis has shown an analogy between specific agglutination and the optimum concentration of H-ions for precipitation of proteins. This latter is constant and characteristic for each protein

and also for agglutination of bacteria by acid. This acid agglutination is quite specific for bacteria so that it is possible to distinguish between typhoid bacilli agglutinated by a H-ion concentration of 4 to 8×10^{-5} , and the paratyphoid, agglutinated by an H-ion concentration of 16 to 32×10^{-5} .¹ As intimated in the preceding sentence, agglutination may be regarded as the precipitation of *particulate* proteins, whereas, *precipitation* in the bacteriological sense, is the precipitation of proteins which are not particulate but which are in solution. With the difference implied in the above sentence, practically everything which may be said for agglutination will be applicable to what is technically known in bacteriology as precipitation. What is said of agglutinins will apply to the precipitins; what is said of agglutinogens will apply to precipitinogens.²

A discussion of the subject of agglutination, no matter how brief, should include reference to the following topics: The use of agglutination in the diagnosis of disease, its use for the identification of bacterial species or strains, the microscopical test, the macroscopical test, group agglutination and agglutinin absorption.

The first and still the most important application of agglutination to the diagnosis of disease is associated with name of Widal. The technic used by him is the microscopical agglutination test and it is routinely employed for the diagnosis of typhoid fever. The Widal reaction was found by Cabot to be positive in 97.2 per cent of 5978 cases studied by him. In 93 per cent of 849 cases it was positive before the eighth day of the disease. Sometimes, however, a distinct positive is not observed until convalescence is well along. In a summary of recent evidence, Gay³ states "that a positive agglutination test may be expected in about 90 per cent of all cases, and that the success in obtaining a positive result increases very markedly from the second week on, until it is positive in nearly all cases by the fourth week."

A small quantity (0.5 to 1 cc) of the blood of the patient is collected and the serum allowed to separate from the clot. One drop of this serum is added to 19 drops of sterile physiological salt solution (0.8 per cent NaCl). This gives a dilution of 1 in 20. Sometimes an ultimate dilution of 1 in 50 is desired. This is easily secured as follows: 1 drop of serum plus 4 drops of sterile physiological

¹ Oertel, Horst.: General Pathology, New York, 1921, p. 135.

² Landsteiner and van der Scheer (Jour. Exper. Med., 1924, **40**, 91) present evidence which casts doubt upon the correctness of this view. They believe that the peculiarities in specificity manifested by precipitinogens and agglutinogens suggest an essential difference in the chemical structures which determine the specificity of the two kinds of antigens.

³ Typhoid Fever, 1918, p. 105.

salt solution. This gives a dilution of 1 in 5. One drop of this plus 4 drops of sterile physiological salt solution gives a dilution of 1 in 25 (*i. e.*, $\frac{1}{5}$ of $\frac{1}{5}$ is $\frac{1}{25}$ or 1 in 25). One drop of this plus the 1 drop of broth culture gives the final dilution of 1 in 50.

A drop of the serum dilution of 1 in 20 or 1 in 25 is placed on the center of a clean coverglass. To this drop is added 1 drop of a vigorously growing, broth culture of a proved typhoid bacillus. This culture has been inoculated less than twenty-four hours previously and has been kept at room temperature (20° C.). The ultimate serum dilution secured by mixing 1 drop of culture with 1 drop of either 1 in 20 or 1 in 25 serum dilution will be respectively either 1 in 40 or 1 in 50. The coverglass is ringed with vaselin or cedar oil and inverted over a concave slide. The examination is made with 4 mm. objective and a 6 or 7.5 ocular.

It is always desirable to run a control with such a preparation. The purpose is to detect any spontaneous, non-specific, agglutination. If such occurs the test must of course be repeated, using either another culture or salt solution from a different flask. The control is simply prepared by adding 1 drop of the same salt solution used as the serum diluent to 1 drop of the same culture, on a coverglass, rimming, inverting over a concave slide and examining alternately with the serum preparation. (Figs. 51 and 52).

A second control is desirable, especially when this microscopical agglutination test is being demonstrated to beginners. In it, it is sought to use the serum of a normal person in place of the patient's. The "normal" person in this case should at least give a definite history of never having had typhoid fever and of never having received prophylactic vaccination against the typhoid bacillus. The serum of such a person is diluted to 1 in 20 or 1 in 25 as in the case of the patient, and a drop of such a dilution and a drop of the broth culture used for the patient's preparation are mixed on a coverglass, rimmed and inverted over a concave slide.

Three such preparations, the patient's, the normal person's, and the control for spontaneous agglutination are made at one time and are examined in series at fifteen-minute intervals for one hour. At the end of that time, both controls should show no essential change either in regard to the motility or distribution of the bacteria from what obtained when the preparations were first set up. If the patient's preparation likewise shows no essential change, the reaction is regarded as a negative one and means that at the time of examination the patient's blood serum had no conspicuous increase in its specific agglutinin content for the typhoid bacillus.

It does not necessarily mean that the patient does not have typhoid infection. That conclusion can only be drawn when the result of the Widal test is taken in consideration with other data.

If, on the other hand, at the expiration of the hour or even at an earlier inspection, the bacilli in the patient's preparation have lost their motility¹ and have gathered together in clumps. (Fig. 50), the reaction is regarded as positive and means an abnormally high, specific agglutinin content for the typhoid bacillus in the serum of this particular patient. This evidence, considered with other data,

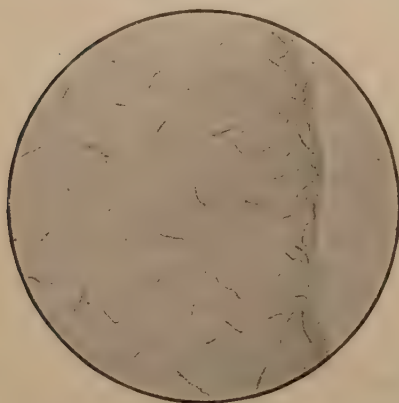


FIG. 51

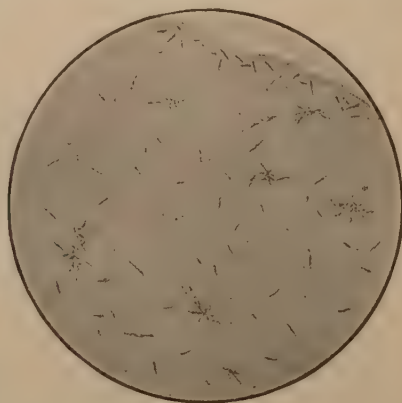


FIG. 52

FIG. 51.—A satisfactory culture for the microscopical agglutination reaction. $\times 430$. This shows a satisfactory culture of the proper density and free of clumps of bacilli. (Twenty-four-hour culture of *Bacillus typhosus* grown at room temperature.) (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

FIG. 52.—An unsatisfactory culture for the microscopical agglutination reaction. $\times 430$. The culture is rather too dense and shows considerable spontaneous or false agglutination of the bacilli. (Twenty-four-hour culture of *Bacillus typhosus* grown at 37°C .) (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

may justify the diagnosis of the disease as typhoid fever. The reason for not using a stronger concentration of serum than 1 in 40 is that experience has shown that the serum of individuals without a present or past history of typhoid may contain enough agglutinins to be demonstrable at stronger concentrations, *e. g.*, 1 in 5 or 1 in 10. Of course, the patient's serum may, and frequently does, agglutinate the homologous organism in much greater dilution than 1 in 50.

In the case just outlined, the Widal reaction, the problem was

¹ Agglutination can occur with non-motile and even killed bacteria. Specific flagellar agglutinins have been described by M. L. Orcutt (Jour. Exper. Med. 1924, 40, 43).

to identify the agglutinins in a patient's serum. These agglutinins are the unknown factor, *i. e.*, unknown as regards their specificity and quantity. The known factor in the solution of this problem was the proved culture of the typhoid bacillus. A known organism was used to detect its homologous agglutinin in concentrations high enough to be clinically significant. The relative concentration of the agglutinin was determined by the method of dilution; the more dilute the solution, *i. e.*, the less serum in a given quantity, which will agglutinate, so much the higher will have been the concentration of agglutinins in the patient's blood plasma. This indicates an important truth valid for all immunity reactions; they are only specific and significant when treated in a quantitative manner.

Identification of Bacteria by Agglutination.—Agglutination is also employed in a way the exact converse of the Widal; such, for instance, as the identification of an organism. The agglutinin content of the serum in this case is the known factor while the organism is the unknown (unidentified) factor. As in the case of the Widal, this use of agglutination is sometimes relied on for the diagnosis of typhoid fever; it is also valuable for the detection of typhoid bacilli in discharges, excretions, soil, foods, and water. To illustrate: A serum of high agglutinin content for *Eberthella typhi* is obtained by the injection (intravenous preferably) of graded, gradually increasing doses of killed and then living cultures of this organism into a rabbit. In this way a serum may be obtained which will specifically agglutinate typhoid bacilli in high dilution, *e. g.*, 1 to 2000 or 1 to 3200 are commonly obtained. The unknown organism whose identity is desired, has been isolated, *e. g.*, on Endo plates, from blood culture or other source. If the microscopical agglutination test is to be used¹ the technic is that of the Widal, namely the ordinary hanging-drop preparation. The rabbit serum with a known, high content of specific agglutinins is mixed with sterile physiological salt solution in a dilution which is twice as concentrated as that which has been shown by preliminary titrations to be critical, *i. e.*, a dilution high enough so the phenomena of natural or group agglutinins with related bacteria will not appear. One drop of such a dilution (twice the critical concentration), is mixed with 1 drop of a broth culture (not over twenty-four hours old and grown at room temperature) of the unidentified organism on a clean coverglass, which after rimming with vaselin or cedar oil is inverted over a concave slide. At least three controls should be run in conjunction: (1)

¹ For this purpose the macroscopic test is probably most frequently used. For the technic of which see below.

Culture of unknown plus the diluent; (2) culture of known organism which is homologous for the agglutinins of the serum plus the serum in the same dilution as that used with the unknown organism; (3) the known organism used in (2) plus the diluent. These four hanging-drop preparations are observed as in the Widal, *i. e.*, every fifteen minutes for at least one hour. Of the controls, 1 and 3 should show no agglutination; while 2 should show agglutination. If the preparation of the unidentified culture plus the serum gives a result at variance with the control (2), *i. e.*, no agglutination, then the conclusion is warranted that the unidentified organism is not identical with the organism used in the control (2). If, on the other hand, agglutination appear in the preparation of unidentified organism plus serum, then the conclusion is warranted that the hitherto unidentified organism is identical with the known organism used in the control preparation (2) (Fig. 50).

This method of identifying bacteria by means of agglutination or other serological tests is regarded at the present time as being the most reliable method available. It represents the court of last resort and possesses a validity superior to all other methods of identification combined.

Macroscopic Agglutination Test.—The technic of agglutination tests just described is known as the microscopic. The macroscopic technic is also frequently used and is regarded as preferable wherever practicable because it permits easier and sharper readings between a serum dilution which will cause agglutination and one which will not cause agglutination. Although the macroscopical technic is used customarily as indicated above for the positive identification of typhoid or typhoid-like organisms obtained in blood culture or from other suspected sources, probably the most frequent clinical employment of this technic is in connection with the "typing" of pneumococci, anticipatory to specific serum treatment.

One-half cc of a homogeneous suspension of the bacteria is added to 0.5 cc of the critical serum dilution in a small test-tube. This critical dilution, it will be remembered, is a dilution high enough to eliminate the complicating effects of natural or group agglutinins, and has been determined in each particular case by a series of preliminary titrations. This mixture is incubated at 37° C. for one hour when the reading is made. Sometimes after this incubation the mixture is kept over night in the refrigerator before the definitive reading is made. Mere naked-eye inspection of the tubes will permit the recognition of agglutination. It is characterized

by a clearing of the suspension of bacteria and their accumulation in visible clumps at the bottom of the test-tubes. A control is indispensable, *i. e.*, a tube containing 0.5 cc of the same bacterial suspension plus 0.5 cc of the sterile physiological salt solution used as diluent throughout the series under consideration. The turbidity of the liquid in this tube should persist practically unchanged. Microscopical examination of the clumps of bacteria at the bottom of a tube will permit one to distinguish between a specific and a non-specific agglutination, *i. e.*, between a true and false positive. In true specific agglutination the clumps are composed of bacteria matted haphazardly together, lying in all directions, while in false non-specific agglutination, the bacteria in the clumps show a distinct tendency to be arranged side by side with their long axes parallel.

The actual details of the macroscopical agglutination test, as given by Avery *et al.*,¹ serve to illustrate the utilization of this method for the identification of a microörganism. The bacteria (pneumococci) in suspension are thrown out by centrifugalization. The bacterial sediment is washed free of culture medium and is taken up in sufficient salt solution to make a moderately heavy suspension, *i. e.*, of turbidity similar to that of a good eighteen-hour broth culture of the pneumococcus. This suspension is mixed with dilutions of immune serum in small test-tubes in equal quantities of 0.5 cc each. The optimum dilutions of serum and the optimum incubation time that will surely identify all type strains and fail to give any cross (group) agglutination reactions, have been determined on a large series of strains. The dilutions given in the following table are such, but might have to be varied when serum from a different source is used.

DETERMINATION OF PNEUMOCOCCUS TYPES BY AGGLUTINATION.

Pneumococcus suspension 0.5 cc.	Serum I (1 to 20) 0.5 cc.	Serum II (undiluted), 0.5 cc.	Serum II (1 to 20) 0.5 cc.	Serum III (1 to 5) 0.5 cc.
Type I	++	—	—	—
Type II	—	++	++	—
Subgroups II a, b, x	—	+	—	—
Type III	—	—	—	++
Type IV	—	—	—	—

Incubation for one hour at 37° C. The double plus (++) means complete agglutination, while the single plus (+) means partial.

As appears in the above table each pneumococcal strain is completely agglutinated only by a serum containing the homologous

¹ Monograph No. 7, Rockefeller Inst. Med. Res., 1917.

agglutinins at the dilutions given. The partial agglutination observed when the atypical Type II pneumococci are mixed with serum containing specific agglutinins for typical Type II pneumococcus, may be regarded as an instance of group agglutination.

Group Agglutination.—The occurrence of this phenomenon must be recognized if one is to have an adequate, general conception of agglutination. Group agglutination is demonstrable in the case of closely related, but antigenically and pathogenically distinct bacteria, such as the typhoid bacillus and the paratyphoid alpha and beta bacilli. Macroscopical agglutination tests were set up, employing pure cultures of paratyphoid alpha and beta and of typhoid bacilli and two sera, one containing specific agglutinins for paratyphoid alpha and the other the agglutinins for paratyphoid beta. The results are tabulated below.

SERUM DILUTIONS.

Organism.	Paratyphoid A.					Paratyphoid B.					Control.
	1/20	1/40	1/60	1/100	1/200	1/20	1/40	1/60	1/100	1/200	
B. typhosus . . .	++	+	+	+	0	++	+	+	+	0	0
B. paratyphosus A	++	++	++	++	++	0	0	0	0	0	0
B. paratyphosus B	+	0	0	0	0	++	++	++	++	++	0

++, complete agglutination; +, partial agglutination; 0, no agglutination.

Inspection of the table shows that each serum had some agglutinating effect upon an organism which was not used in the preparation of the agglutinating serum. Both paratyphoid alpha and paratyphoid beta sera produced some agglutination of typhoid bacilli even in a serum dilution of 1 to 100. This is an instance of group agglutination. The common interpretation of this phenomenon is that the bacterial cell of each species (or strain or type) consists of a number of proteins, each of which is capable of acting as an agglutigen. Corresponding to each agglutigen there would be in the specific serum a distinct agglutinin. This can be represented symbolically. Let the agglutinogens of species X be A, B, C, T; of species Y be A, C, D, S. The immunization of an animal with bacterium X will result in a serum rich in the following agglutinins, *a, b, c, t*; while when Y is used the serum will be rich in the agglutinins *a, c, d, s*. It is obvious that if such be the case, then a serum specifically agglutinating bacterium X will also, by

virtue of the agglutinins, *a* and *c*, be capable of exerting some agglutinating action upon bacterium *Y* because this organism contains the agglutinogens, *A* and *C*.

Absorption of Agglutinins.—Although the simple quantitative relations observed in elementary chemistry, as the neutralization of acids by alkalis, do not govern the antigen-antibody reactions of immunity, these latter nevertheless obey definite quantitative laws. The antibodies in a serum can be removed from that serum by saturation with the homologous antigens. Advantage is taken of this fact in distinguishing between specific and group agglutination. If a monovalent serum agglutinates two bacterial species, the saturation of this serum with the homologous organism will practically remove the agglutinating power for other organisms. On the contrary, saturation with an organism which is not homologous will cause practically no loss of agglutinating power for the homologous organism. The following table from Castellani¹ illustrates this.

SERUM OF RABBIT IMMUNIZED WITH TYPHOID BACILLI ONLY.

	Initial agglutinating titer of serum.	After absorption with typhoid bacilli.	After absorption with colon bacilli.
Typhoid bacilli . . .	1 to 10,000	No agglutination	1 to 10,000
Colon bacilli . . .	1 to 800	No agglutination	No agglutination.

The agglutination of the colon bacilli in serum dilution of 1 to 800 is due to the group agglutinins. The failure of this serum after saturation with the homologous organism (typhoid) to agglutinate colon bacilli shows that the initial agglutination of these organisms was due to the presence of group agglutinins.

The test for absorption of agglutinins is not difficult. The agglutinating serum is mixed with an excess of the organism in question and incubated. The temperature and time of incubation varies somewhat with the organism used. At the expiration of this incubation period the mixture is centrifugalized at high speed, to throw all the organisms out of suspension. The clear supernatant serum is then used to make up various dilutions with which are performed in the usual manner macroscopic agglutination tests with the homologous and other bacterial species in question. Beside the usual control of bacterial suspension plus diluent, it is essential to have another control series in which the serum alone, without bacteria, has been incubated under the same conditions to which was

¹ Ztschr. f. Hyg., 1902, vol. 40, quoted by Zinsser, *Infection and Resistance*, 1923, p. 255.

exposed the mixture of serum and bacteria. Agglutinin absorption is in brief a test devised to show the removal of the specific agglutinins from a serum.

Significance of Agglutination.—The full significance of agglutination in the defense of the body against bacterial invasion is not yet known. Agglutination *per se* does not appear to injure the viability or pathogenicity of the affected bacteria. It has even been suggested that the formation of agglutinins is rather an incidental and protectively unimportant accompaniment of other reactions. There is, however, very convincing evidence that such is not at least always the case. Bull¹ employed pneumococci, typhoid, dysentery and influenza bacilli injected into rabbits. His studies showed that in both passive and natural immunity, the agglutination of the bacteria within the blood of the infected animal was followed by their rapid removal from the circulation. In the visceral capillaries these clumps of bacteria were phagocytosed and destroyed. On the contrary, bacteria which did not undergo a preliminary agglutination, remained in the circulation and produced a progressive septicemia. The agglutinins seem at least to interfere with the free dispersion of bacteria and to that extent mechanically facilitate phagocytosis.

Blake² believes he has evidence that the degree to which pneumococcal metabolism is inhibited by antipneumococcal serum is in proportion to the agglutinating power of that serum. The formation of methemoglobin, which is easily recognized by a color change in the medium, may be used as an index. It is to be remembered that the formation of methemoglobin from hemoglobin is a normal and conspicuous function of pneumococcal metabolism.

It is seen that where agglutination is complete, no methemoglobin is formed and conversely that where there is no agglutination the formation of methemoglobin is complete. Between these two extremes there is the graded series to be expected from Blake's hypothesis.

The inhibition of metabolism illustrated above apparently extends to involve the reproductive capacity of the bacteria, a function of the highest significance for pathogenicity. The mechanism of this inhibition may merely lie in interfering with the intimate contact of the bacterial cell with the whole medium. The following table illustrates the marked inhibition of growth exerted by antipneumococcal serum upon the homologous organism, and Blake regards this inhibitory action as a function of the agglutinin content.

¹ Jour. Exper. Med., 1915, 22, 484.

² Ibid., 1917, 26, 563.

Culture, 0.5 cc.	Serum, 0.5 cc.	Agglutination after 2 hrs. at 37° C.	Methemoglobin formation after 1 hr. at 37°C. ¹
Pneumococcus Type I	Antipneumococcus Type I (1 to 25)	++	—
Pneumococcus Type I	Antipneumococcus Type I 1 to 50)	++	± ±
Pneumococcus Type I	Antipneumococcus Type I (1 to 100)	+	± ±
Pneumococcus Type I	Antipneumococcus Type I (1 to 200)	±	± ±
Pneumococcus Type I	Antipneumococcus Type I (1 to 400)	—	++
Pneumococcus Type I	Antipneumococcus Type II 1 to 25)	—	++
Pneumococcus Type I	Normal horse serum (1 to 25)	—	++

± ± indicates methemoglobin formation in the immediate vicinity of the agglutinated pneumococci, the upper portion of the medium remaining unchanged.

Culture, 10-8 cc.	Number of colonies.				
		Imme- diately.	After 3 hrs.	After 6 hrs.	After 24 hrs.
Pneumococcus Type I	Antipneumococcus Type I	61	54	80	Confluent.
Pneumococcus Type I	Antipneumococcus Type II	50	45	12,000	"
Pneumococcus Type I	Normal horse serum	37	284	22,000	"

Equal samples of the culture-serum mixtures indicated in the first two columns were plated out at the intervals indicated in the four columns at the right. After incubation the number of colonies developing were counted and noted. It is obvious that if the differences observed in the number of colonies are largely due to the effects of the agglutinins in the serum, then these antibodies are of great value in the resistance of the host.

Gay² has noted that a high Widal titer is of good prognostic significance in typhoid fever. It has been suggested that the agglutinin content of a serum is chiefly of value as indicating the degree of the defensive reaction. Garbat,³ however, has observed that there is no direct correspondence between the complement-fixation and the agglutination tests during the acute stage or convalescence of typhoid fever. This fact, taken together with that mentioned by Gay, favors the view that some intrinsic, immunological value attaches to the agglutinins.

¹ One cubic centimeter hemoglobin solution added to each tube.

² Typhoid Fever, New York, 1918, p. 229.

³ Loc. cit., p. 92.

Ottenberg¹ has reported that phagocytosis of foreign red cells in the circulation after blood transfusion occurs only when the patient's serum has an agglutinative action on the donor's cells.

Fenn² has observed that when a suspension of quartz and carbon particles is offered to leukocytes, the carbon is ingested about four times as readily as the quartz. It is suggested that this result is determined by the greater instability of the carbon suspensions. Similarly, manganese dioxid is ingested by leukocytes with extraordinary rapidity compared to manganese silicate, which latter forms a much more stable suspension. The bearing of these observations on the phagocytosis of bacteria is to confirm the view that bacteria are more readily ingested when easily agglutinated, *i. e.*, *when combined with agglutinin*.

The evidence presented by Bull, Blake, Gay, Ottenberg and Fenn favors the view that agglutinins are something more than a mere by-product of the defensive reaction and that they possess an intrinsic, protective importance.

ANTITOXINS.

So far we have considered the properties or substances of the serum or other body humors which exert their effect upon the microorganism itself. Attention will now be turned to the properties or substances of these body humors which tend to neutralize, nullify, or destroy the toxic *products* of the microorganism, but which leave the microorganism itself unaffected. In the first place it may be said that as yet no serum property or constituent of the host, operative against an endotoxin, has been successfully demonstrated. Consequently we shall at once take up the *antitoxins*. This term has been applied to that property or constituent of the body humors of the host which neutralizes and renders inert the true toxins or exotoxins. The toxin molecule by its union with antitoxin is at least for a time not destroyed. Toxin, under certain conditions, can be recovered from previously non-poisonous toxin-antitoxin mixtures. Antitoxins are produced against the toxins of the diphtheria bacillus, the tetanus bacillus, *Clostridium botulinum*, the bacillus of symptomatic anthrax, the bacilli of gas gangrene, Shiga's dysentery bacillus and *Pseudomonas aeruginosa*. Antitoxins have also been prepared against snake venoms, arachnotoxin, eel serum, abrin, ricin, and crotin. The

¹ Quoted by Zinsser, *Infection and Resistance*, New York, 1918, p. 239.

² Phagocytosis of Solid Particles (III), *Jour. Gen. Physiol.*, 1921, **3**, 575.

neutralizing effect of antitoxin is specific, *i. e.*, the antitoxin produced against the toxin of the diphtheria bacillus will neutralize this toxin, but will be completely inert toward the toxin of the tetanus bacillus or any other microorganism.

Nothing is known with certainty about the chemical nature of antitoxins. In this respect they do not differ from the toxins or any of the peculiar defensive bodies of the humors. The antitoxin molecule or complex is apparently much larger than the toxin molecule or complex. Antitoxic solutions behave in all respect as colloids. When the globulins are precipitated from antitoxic sera the antitoxins are carried down with the precipitate. The antitoxins seem to be particularly associated with the pseudoglobulin fraction. Heating to 60° or 70° C. diminishes and boiling destroys their efficacy. On the whole, Wells (1920, p. 177), concludes that "the evidence indicates a closer resemblance of antitoxins to proteins than has been shown for the toxins, and all attempts to separate antitoxins from proteins have so far failed."

Toxin and its corresponding antitoxin combine in definite proportions. The reaction, whatever be its nature, is quantitative. A definite quantity of toxin is neutralized by a definite quantity of antitoxin. If the quantity of toxin be represented by T and the equivalent quantity of antitoxin by A , then $2T$ will require $2A$, $3T$ will require $3A$, etc. nT will require nA . These relations hold when all the requisite antitoxin is added at one dose to the toxin, but they do not hold when the quantity A is added in several fractions, with a certain time interval between successive fractions. Ehrlich early observed that in general the first part of the antitoxin added neutralizes, generally speaking, a greater portion of the toxin than does the second addition of an equal amount of antitoxin, that this second fraction neutralizes a greater amount of toxin than does the third addition of an equal amount of antitoxin, and so forth. This phenomenon can be stated more concretely. If a given quantity of antitoxin, A , be divided into three equal parts, A_1 , A_2 , and A_3 , and if these be added successively at appropriate intervals to a quantity of toxin, it will be found that the addition of A_1 will occasion a much greater drop in the toxicity than will follow the addition of A_2 , and that the addition of A_2 will occasion a greater drop in the toxicity than will follow the addition of A_3 . This phenomenon in the neutralization of toxin by its corresponding antitoxin can be graphically represented by the accompanying curve (Fig. 53). The values along the base line represent the quantities of antitoxin used. The length of the perpendicular at the

extreme left between the base line and the point of origin of the curve, represents the original toxicity before the addition of any antitoxin. The length of a perpendicular erected from any point on the base line, delimited by this line and by the point of intersection of the perpendicular with the curve, indicates the amount of residual toxicity demonstrable in the toxin-antitoxin mixture at a given stage of the "neutralization."

Three principal hypotheses have been proposed to account for the nature of the toxin-antitoxin reaction, *i. e.*, to put it simply, to account for this type of curve. We shall designate them by the names of their most prominent protagonists: (1) Ehrlich's; (2) Arrhenius' and (3) Bordet's.

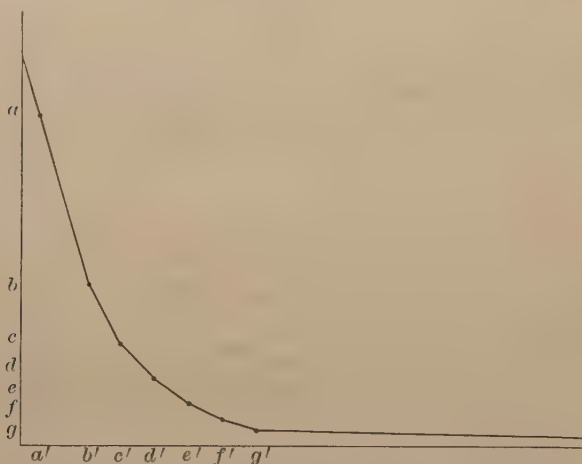


FIG. 53.—The neutralization of tetanolsyn by antitoxin. The toxicity of the mixture drops as antitoxin is added. (After Arrhenius.)

1. The use of the term "neutralization" to designate this reaction carries with it the implication that we are here dealing with a process closely analogous to the neutralization of a strong acid by a strong base. This was essentially the view of Ehrlich. As a matter of fact, however, the "curve" indicating the course of such a genuine neutralization is a straight line (Fig. 54). Ehrlich and his associates, of course, were aware of this discrepancy. In order to overcome it, *i. e.*, in order to harmonize the type of curve of a genuine toxin-antitoxin union with their view that the toxin-antitoxin reaction was in the nature of the neutralization of a strong acid by a strong base, they were forced to assume that the toxic solution was in reality a mixture containing such bodies as toxin, toxoid, prototoxin,

deuterotoxin, tritotoxin, epitoxin and toxon, of varying toxicities and affinities for antitoxin. Ehrlich's "explanation" is very complicated and we may at once dismiss it. It is a canon of science to choose of two or more alternative, equally satisfactory hypotheses, the simplest. Further it is methodologically preferable, if phenomena can be "explained" in terms of an already established law of wide applicability, to adopt such a law rather than to invent an "explanation" *de novo*.

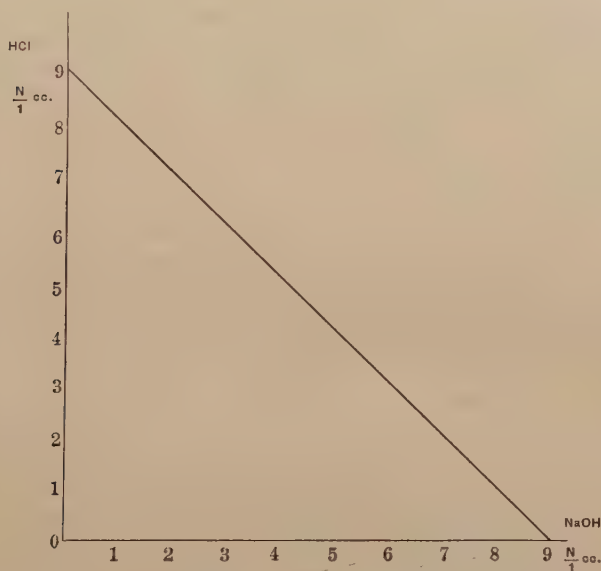


FIG. 54.—Curve of neutralization of a strong acid by a strong base. Note that the addition of each cubic centimeter of the base neutralizes exactly the same amount of the acid as is neutralized by any other cubic centimeter of the base.

It is unlikely, judging by chemical analogies, that the toxin combines with antitoxin as a strong acid, *e. g.*, HCl, reacts with a strong base, *e. g.*, NaOH. Both toxin and antitoxin are of biological origin, probably belonging to the group of organic compounds. If they are not actually colloids they have so far proved themselves to be inseparably associated with colloids. And colloids do not interact as strong acids and strong bases. The rate of reaction for strong acids and bases is very rapid; but the reaction of toxin and antitoxin requires an appreciable time for its completion. This circumstance also obtains for many reactions between organic compounds.

2. Arrhenius and Madsen in a series of valuable investigations have called attention to an interesting fact. If we represent by a curve the reaction of ammonia and boric acid (Fig. 55) we secure a graph which closely resembles that which we have seen above holds for the "neutralization" of a toxin and antitoxin. This type of curve is frequently encountered in the chemical reactions of organic compounds. In fact, all reactions which conspicuously illustrate the Law of Mass Action of Guldberg and Waage afford data which when graphically represented, describe a curve of this type. This law is of fundamental importance in chemistry and deserves some

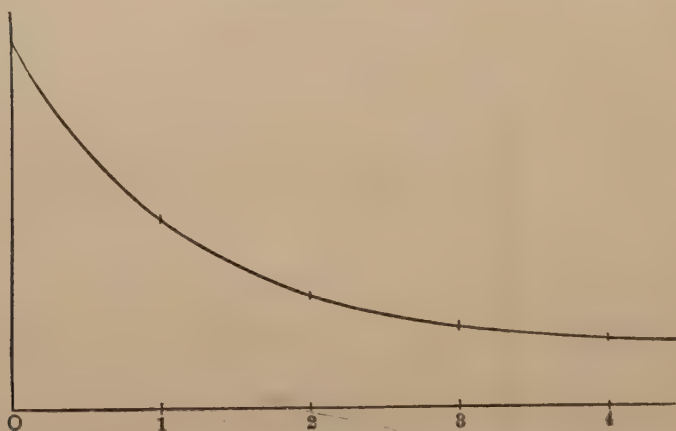


FIG. 55.—Curve of hemolysis by ammonia during progressive neutralization by addition of constant quantities of boric acid. (Wells.)

elaboration. The equation for the reaction of acetic acid and ethyl alcohol is written as follows:



The arrows, pointed in opposite directions, which are used in place of the sign of equation ($=$), indicate that the reaction is reversible. When the reaction is "completed," *i. e.*, when equilibrium is established, we find not only the two compounds at the right of the equation but we find all four compounds present. The quantities present upon the establishment of equilibrium bear a very definite relationship to one another. A change in the quantity of any one of the four compounds will disturb the equilibrium, so that the reaction will be reinitiated and will continue until a new equilibrium is

established. The quantitative relations of the reacting compounds are represented by a very simple formula:

$$a b K_1 = c d K_2$$

$$\frac{a b}{c d} = \frac{K_2}{K_1} = K$$

a = the *active* quantity of CH_3COOH .

b = the *active* quantity of $\text{C}_2\text{H}_5\text{OH}$.

c = the *active* quantity of $\text{CH}_3\text{COOC}_2\text{H}_5$.

d = the *active* quantity of H_2O .

K and K_2 factors, experimentally determined, peculiar to a given reaction at a given temperature. The value K is a constant which holds when equilibrium has been reached among the reacting substances, no matter what may have been their original, absolute quantities.

It is obvious that an arbitrary change in the value of any one of the reacting substances, *e. g.*, in c , will necessitate a compensatory change in the value of all the other reacting substances, *e. g.*, a , b , and d , if the value K is to be maintained unchanged. In this way it is possible to change the direction of the reaction from that of left to right to that of right to left, as indicated in the above "equation." This phenomenon of reversibility, is exhibited by a great variety of the reactions of organic chemistry, and is an essential characteristic of the Law of Mass Action of Guldberg and Waage. In chemical reactions of this nature, no matter how much time has passed since the establishment of a state of equilibrium, the property of reversibility persists undiminished. It is at any time possible, by appropriately altering the active concentrations of the reagents, to induce a reaction whose direction will be opposite to that which had resulted in the establishment of the equilibrium.

The fact which most strongly indicates that the "neutralization" of toxin by antitoxin is not a particular case of the Law of Mass Action is that the property of reversibility is gradually lost. Although for certain periods of time it is possible to recover toxin or antitoxin from toxin-antitoxin complexes (wherefore we speak of a "neutralization" rather than a destruction of the toxin molecule), this possibility does not hold indefinitely. The union of toxin and antitoxin seems to become progressively stronger and more *stable*, until finally no "dissociation" is possible. Neither toxin nor antitoxin can be recovered from toxin-antitoxin mixtures which have stood for some time.

3. A third hypothesis (we have already referred to the hypotheses of Ehrlich and Arrhenius) has been suggested by Bordet. Accord-

ing to this view the "neutralization" of toxin by antitoxin is regarded as a phenomenon of *adsorption*. Common examples of adsorption are the concentration of hydrogen on the surface of finely divided platinum as utilized in many types of patent gas lighters, or the concentration of a dye on a fabric as seen in many commercial dyeing processes. The effects of adsorption are easily demonstrated by adding ordinary table salt (NaCl) to a solution of soap. A certain point is reached when the soap is precipitated out of solution. This is interpreted as a sequel to the concentration of the NaCl or its constituent ions upon the surface of the minute particles of soap in colloidal solution, *i. e.*, the particles of soap, of ultramicroscopical dimensions, *adsorb* the electrolyte or its ions. Similar adsorption phenomena are common in colloid chemistry and are very important for the understanding of many physiological and biological processes.

If the "neutralization" of toxin by antitoxin be an adsorption phenomenon, then the loss in toxicity exhibited by a toxin solution following the addition of antitoxin would be directly proportional to the amount of antitoxin adsorbed. In other words, the residual toxicity in toxin-antitoxin mixtures is inversely proportional to the amount of antitoxin combined with the toxin. If the curve given above illustrating the "neutralization" of tetanolyisin by antitoxin, be transposed in accord with this assumption, we secure a type of curve which is constantly encountered in all work on adsorption. The assumption is justified because it is in the very nature of the adsorption hypothesis. The transposition is easily effected by inverting the order of the values indicated on the perpendicular at the left of that graph. In going up from the base line, instead of having increasing toxicities, one now has in the transposed curve (Fig. 56), increasing amounts of antitoxin that is combined with toxin. Such a curve indicates that *relatively* more antitoxin combines with a given quantity of toxin from more dilute solutions of antitoxin than from more concentrated solutions of antitoxin.

This same type of curve is exhibited by many reactions which are acknowledged to be adsorptive in nature, *e. g.*, Fig. 57.

From the above paragraphs it appears that the processes: (1) Of the neutralization of a weak acid by a weak base (Law of Mass Action); (2) of the adsorption of one substance by another; (3) of the fractional "neutralization" of toxin by antitoxin, can all be graphically represented by one and the same type of curve.

The adsorption hypothesis of Bordet appears preferable, however, to hypothesis of Arrhenius (Law of Mass Action) at least in that the complexes, resulting from adsorption, though at first "disso-

ciable" into their constituents, nevertheless eventually may lose their "dissociability." That is, the process by which the adsorption

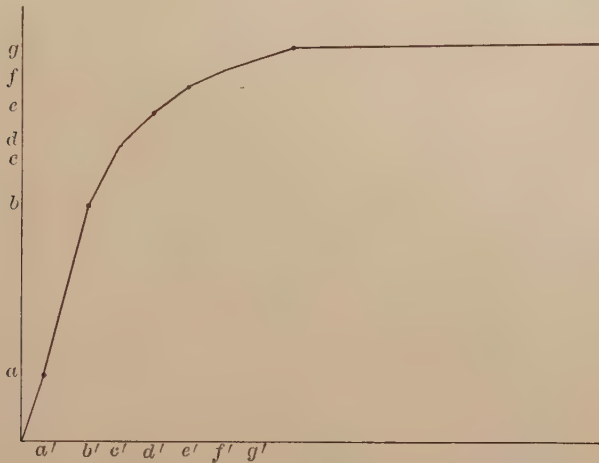


FIG. 56.—"Neutralization" of tetanolysin by antitoxin. This curve was constructed by transposition from that in Fig. 53. Notice that the difference lies in reversing the order of the values ($a \dots g$) at the left. This is done on the assumption that the residual toxicity is inversely proportional to the amount of antitoxin "adsorbed" with toxin.

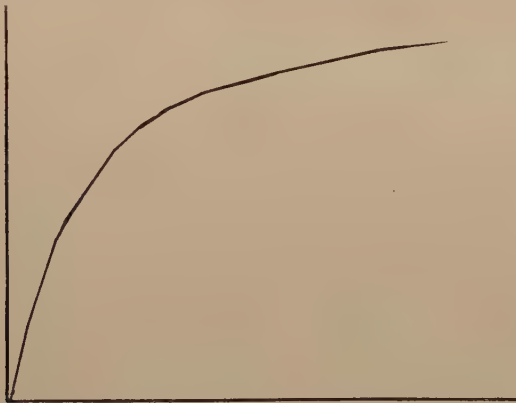


FIG. 57.—Compare with Fig. 56. Typical adsorption curve, constructed on data contained in Millard's Physical Chemistry for Colleges, p. 108. Adsorption of nitrogen by a given quantity of mica at 90° absolute. Increasing quantities of nitrogen offered are represented on the base line, starting at the point of intersection and going to the right. The distance above the base line, of a point on the curve represents the amount adsorbed at that particular concentration of nitrogen. Note that the relative amount adsorbed decreases rapidly with increasing concentration.

complex is formed may become irreversible. This is exactly what is observed in the case of mixtures of toxin and antitoxin,

It has been urged against the adsorption hypothesis that it does not account for the marked specificity which is seen in the case of toxin "neutralization" by antitoxin. As a general rule, the process of adsorption is possibly not as specific as the classical reactions of elementary inorganic chemistry. But we must remember that even in this latter field, one compound will react with many others and consequently specificity is not absolute. It is also true that cases of marked specificity in adsorption phenomena are known. It is certainly premature to rule Bordet's hypothesis out of consideration because of any alleged lack of specificity in adsorption reactions.

Another objection has been raised against Bordet's hypothesis. The attraction of one colloid for another has often been interpreted solely on an electrical basis. It is known that colloid particles carry electrical charges and the attraction between two colloids has been regarded as the expression of the attraction of two oppositely charged particles. It is known that toxin and antitoxin both behave as if carrying a positive charge.¹ If the possession of opposite electrical charges were a necessary requisite for the interaction of two colloids, then, of course, the toxin-antitoxin reaction could not be a reaction of this nature. As a matter of fact, however, it is known that colloids can react in this way even though they be carrying charges of the same sign.

In summarizing this discussion of the nature of the "neutralization" of toxin by antitoxin, it may be said that the evidence available today favors the view proposed by Bordet. The curve of the fractional "neutralization" of toxin by antitoxin is like the curve describing the mutual adsorption of two colloids. The resulting complex, though at first "dissociable," in time loses this "dissociability." It has been shown that the objections: (1) That adsorption is not specific enough, or (2) that two colloids to react mutually must carry electrical charges of opposite signs, are not always valid when applied to the adsorption phenomena of colloids. It seems likely that at least the first step in the "neutralization" of toxin by antitoxin is governed by the laws of adsorption. It is, of course, possible that after the formation of such a primary adsorption complex, chemical forces in the narrower sense of the word become operative and a genuine chemical compound may result.

A clearer conception of what occurs according to Bordet when antitoxin is added to toxin, may be gained by the employment of definite figures. The values given below are, of course, chosen

¹ Field and Teague: *Jour. Exper. Med.*, 1907, **9**, 86.

arbitrarily. Let us assume that we have 10 portions of toxin (T) and 10 equivalent portions of antitoxin (A). If to 10T we first add 1A, we will not have 1(T+A) compound formed with 9 portions of T left unaffected, as would happen if neutralization in Ehrlich's sense occurred. Instead of this the portion 1A will be distributed by adsorption so that each portion of the 10 portions of T will be associated with 0.1 portion of A. If then to such a mixture a second portion of 1A be added, this will be evenly distributed among each of the 10 portions of T, so we shall have in the mixture 10 adsorption complexes each of which can be represented as $(T + 0.2A)$. Each successive addition of the antitoxin will be distributed in a similar fashion *pro rata* among the 10 portions of toxin. In this way we shall have a progressive loss of toxicity in the toxin solution.

Attention to the reverse of this phenomenon has been called by Danysz. "When an excess of toxin is added to its specific antitoxin in several portions at proper intervals of time," the residual toxicity of the mixture is much greater "than if the same quantity of toxin had been added to the same quantity of antitoxin at one time." This "Danysz effect" is also susceptible to interpretation as a process of adsorption.

The views of Bordet have been applied not only to the toxin-antitoxin reaction, but also to all the other immunity reactions, such as the phenomena of bacteriolysis, agglutination, precipitation and opsoninization.

The disappearance of agglutinin from serum, when various concentrations of the serum are added to a given quantity of the homologous bacterium, has been carefully studied by Dreyer and Douglas. A curve representative of their results is given in Fig. 58. Increasing concentrations of agglutinin are given on the base line, beginning at the left. The distance of the curve above the base line indicates the amount of agglutinin which has combined with the bacterium. Although Dreyer and Douglas did not feel that their data conclusively showed that agglutination was an adsorption process, the similarity of the first part of this curve to that illustrative of an unquestionable case of adsorption (nitrogen and mica) is striking. The gradual decrease of the quantity of agglutinin combining with the bacterium, which appears after a certain concentration of agglutinin has been overstepped, is a feature which has not come to light in the above discussion. Dreyer and Douglas suggest that it is caused by "some obscure alterations in the surface tension due to change in the concentration of the albumen or of the different salts, or in the viscosity of the fluid, etc." This peculiarity of this

curve does not eliminate the possibility that agglutination may be an adsorption phenomenon. The authors called attention to the fact that in a study of the adsorption of Congo red by filter paper, Bayliss showed that the amount adsorbed not only reaches a maximum but even decreases subsequently.

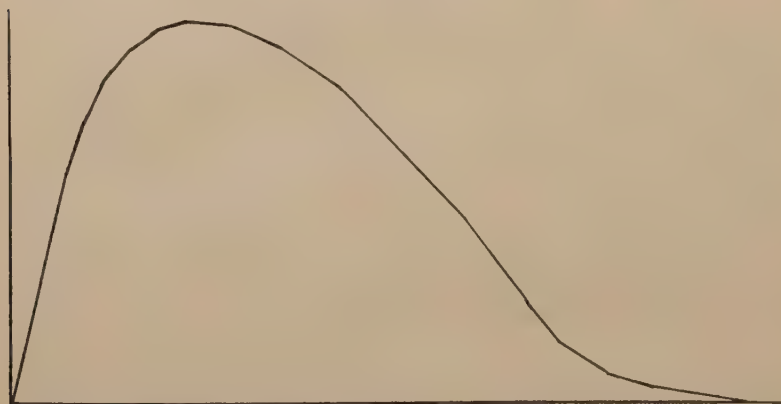


FIG. 58.—Agglutinin-agglutinin reaction. Curved based on data given by Dreyer and Douglas (Proc. Roy. Soc., 1910, **82**, 185, Table I, p. 192).

In concluding it should be pointed out that according to modern concepts¹ the process of adsorption is not so distinct from the classical reactions of elementary chemistry or from those reactions clearly illustrating the Law of Mass Action, as was thought to be the case when Bordet and Arrhenius first proposed their hypotheses. It has been recognized that in most cases, denominated as adsorption, chemical affinities play a part, and with increasing knowledge the possibility of setting adsorption phenomena apart as distinct from chemical phenomena seems to become more and more remote. This is just a particular case of the current tendency to regard the laws of colloid chemistry not as essentially different from those of ordinary chemistry. As an instance contributing to, and exemplifying this attitude, may be cited the latter work of Jacques Loeb on the proteins.

It is easily conceivable that the hypotheses of Arrhenius and of Bordet are not really contradictory or mutually exclusive. They both may importantly contribute to a broader, more comprehensive and more satisfactory hypothesis of the nature of immunity reactions.

¹ Mathews: *Physiol. Rev.*, 1921, **1**, 553. Bayliss: *The Colloid State*, Oxford Med. Pub., 1923.

EHRlich'S SIDE-CHAIN THEORY.

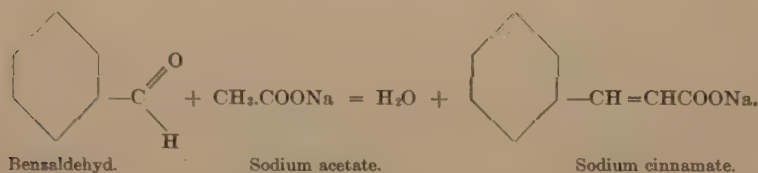
The word *antibody* has come into general use to designate one of the hypothetical substances in the body humors, participating in the defense against infection. For example, an antitoxin is an antibody, and so are the agglutinins, precipitins, amboceptors, complement and opsonins. The substance which reacts specifically with the antibody and which occasions the production of antibodies, is known as an *antigen*, *i. e.*, a former or generator of antibodies. The several toxins are antigens, and so are the constituents of the microorganism which determine the production of agglutinins, precipitins, amboceptors and opsonins. The antigen which occasions the production of an agglutinin, is termed an agglutino-gen; one which occasions the production of a precipitin, is termed a precipitinogen. Other substances than microorganismal constituents or metabolites can act as antigens; *e. g.*, the various native, coagulable proteins (serum and egg-white), abrin, croton, ricin, eel serum, arachnotoxin, snake venoms, etc.

The mechanism by which antibodies are formed and the nature of the union of antigen with antibody are unknown. The most interesting and best known theory which deals with these questions is that known as the side-chain theory of Ehrlich. Before considering this subject more in detail it will not be amiss to point out the great importance of the formulation of theories in scientific work. A scientific theory has two chief functions: (1) It proposes a correlation of otherwise isolated, disconnected "facts" into a harmonious, rational system; and (2) it serves as a stimulus and guide for further investigation. In other words, every theory is only a "working" hypothesis. To make little of the theoretical phases of a science can only be done by one who does not know or who does not appreciate the salient features in the development of human knowledge. In the words of Cardinal Newman, "Theories speculations, hypotheses, are started; perhaps they are to die; still not before they have suggested ideas better than themselves. These better ideas are taken up in turn by other men, and, if they do not lead to truth, nevertheless they lead to what is still nearer the truth than themselves; and thus knowledge on the whole makes progress. The errors of some minds in scientific investigation are more fruitful than the truths of others." Even judged by ultimate material usefulness, nothing is really more practical than a scientific theory, legitimately formed and intelligently tested. It should not be forgotten, to cite one striking example, that the discovery of the

Western hemisphere accidentally and unexpectedly resulted from action premised on the *theory* of the sphericity of the Earth.

A satisfactory comprehension of Ehrlich's side-chain theory will be made easier by pointing out what ideas it fundamentally involves. In the first place it is based on a chemical analogy. It is a mistake to forget that it is an analogy and to take the chemical terminology too literally. In the second place it makes use of a concept enunciated by Weigert¹ to the effect that at times reparative processes continue at work even after the damage which started them, has been made good. As common examples of this phenomenon are the formation of "proud flesh" and the production of those peculiar neoplasms called keloids, at the site of wounds. In the third place, Ehrlich's theory is particularly interesting because it regards the way by which an "antigen" injures a cell as not essentially different from the way by which food is assimilated by that cell. This is in harmony with a pathological generalization of long standing that for many (if not all) pathological processes there exists a physiological analogue. Possibly this idea can be stated in broader terms by saying that the body reactions in disease no less than in health are the legitimate subject matter of biology.

According to Ehrlich's hypothesis the cell may be considered to consist chemically of a "nucleus" (not to be confused with the morphological nucleus), extending out from which are a number of radicals. The nature of the chemical "nucleus" determines the essential nature of the cell, while the peripherally attached radicals serve to establish communication between this chemical "nucleus" and the chemically reactive substances in the environment of the cell. To employ an analogy from organic chemistry, the following reaction may be given:



The benzene hexagon of benzaldehyd represents the chemical "nucleus" of the cell. The aldehyd radical is the means by which the sodium acetate combines with the benzaldehyd molecule. In chemical language the radical attached to a larger, central ring

¹ Verhandl. d. Ges. deutsch. Naturf. u. Aerzte (68 Vers, Frankfurt a. M., Erste Theil, Allgemeine Sitzungen), 1896, p. 121.

or group, is known as a side-chain. The aldehyd group is such a side-chain; and through it the sodium acetate "exerts an influence" upon, and so modifies, the benzaldehyd that there results the sodium cinnamate molecule. The processes of normal cellular assimilation were pictured by Ehrlich as possibly analogous. Of course, in the case of the cell, the processes are inconceivably more complicated.

Extending peripherally from the chemical "nucleus" is a great variety of side-chains, having varied affinities for the different molecules that float past in the blood, lymph or tissue juices. It is by virtue of these side-chains that the cell can combine with and utilize the various substances that are necessary for its functions, growth, repair, and energy requirements. Presumably a particular kind of side-chain may combine with the oxygen, and in a like fashion for each of the ultimate, assimilable products of proteins, carbohydrates, and fats there may correspond a particular kind of side-chain. The various hormones which influence the cell do so presumably in terms of Ehrlich's hypothesis through the mediation of various side-chains, between which and the hormones there exist chemical affinities.

Now it would not be strange if from this multiplicity of side-chains, some did exist which by mere chance had affinities for substances which did not normally present themselves in the course of metabolism. And it is also easily conceivable that some of such unusual substances might after combining with their corresponding side-chains, occasion functional or morphological disturbances in the cell. This disturbance is, of course, the essence of disease and the property of occasioning this disturbance would be toxicity.

It is self-evident from the view-point of Ehrlich's theory that a foreign, unusual substance if no affinities existed between it and the side-chains, would be perfectly innocuous to the cells. It is therefore possible to regard some instances of natural insusceptibility to certain infections, as explicable on this basis. If the cells of an animal possess no side-chains which can enter into combination with the constituents or metabolites of a particular microörganism, then this microörganism is non-pathogenic for that animal. It may also be pointed out at this time that even if a foreign, unusual substance, not normally presented in the course of metabolism, did by virtue of combinability with a certain kind of side-chain possess potential toxicity, this substance might nevertheless be rendered innocuous. This detoxication evidently would result if the side-chain in question existed in sufficient numbers free, that is detached from its parent cell, in the blood plasma or other body

humor. Such free side-chains would be in a position to combine with all the molecules of the potentially toxic substance before this could come in contact with the cells. In this way any deleterious influence on the body cells would be avoided and disease sidetracked.

Ehrlich regarded the antitoxins, agglutinins, precipitins and amboceptors of immune sera, to be such side-chains. The production of a particular kind of side-chain in large numbers and their liberation from the parent cell into the body humors, constitute the primary problems of immunity according to Ehrlich's theory. The different kinds of side-chains and the manner in which they render innocuous the potentially toxic foreign substances are other aspects of this theory which have been extensively elaborated.

A more vivid idea of this theory can be gained by describing the series of events in an imaginary case. An unusual substance of bacterial origin (antigen), capable of injuring a cell, is present in the blood. Through a particular type of side-chain it combines with a cell. This action *per se* destroys the side-chain as such. In the cellular reaction that follows, that particular type of side-chain is reformed. The regenerative process does not stop when the *status quo ante* has been reestablished but, according to Weigert's law continues, resulting in the production of an excess of the type of side-chain destroyed. As equilibrium is reached, the excess is cast off into the body humors. These free side-chains can combine with additional quantities of the antigen which was responsible for the initiation of this process. In this way the antigen can be diverted from the susceptible cells and disease be averted.

Side-chains of this nature were called by Ehrlich *receptors*. He recognized three fundamental types or orders of receptors, *viz.*, receptors of the first order, of the second order and of the third order. Because their chemistry is unknown it is customary to represent them graphically by symbolic diagrams, such as are used in the following discussion. These symbols are purely arbitrary and must not be taken in any sense as an approximation of the form and appearance of the antigens or antibodies involved.

Receptors of the First Order.—These are the antitoxins. The nature of toxin and antitoxin and the manner of their union are symbolized in the accompanying figure (Fig. 59). There is evidence that the toxin molecule consists of two parts; one through which it combines with the side-chain or antitoxin and the other part in which resides its toxicity. The term *haptophore* is applied to the combining group, while the term *toxophore* is applied to the poisonous group or radical within the molecule. The fact that solutions

of toxin on standing readily lose their toxicity although they still retain much of their ability to bind antitoxin, suggests this view of the constitution of the toxin molecule. The term *toxoid* is applied to the substance which binds antitoxin but which is not poisonous. As appears from the diagram the antitoxin molecule may be regarded as a simple *haptophore* group combining specifically with toxin. This conception reflects the fact that active toxin may be recovered in an apparently unaltered condition from neutral mixtures of toxin and antitoxin. If antitoxin not only combined

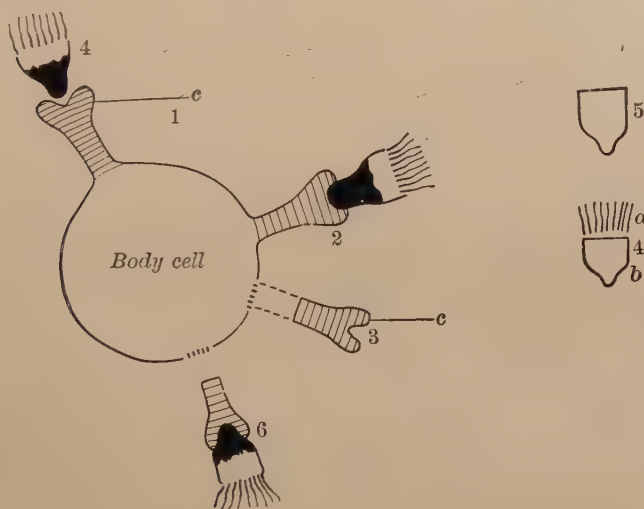


FIG. 59.—Side chains, first order (antitoxins and antiferments). 1, side chain attached to cell; *c*, haptophore group; 2, side chain to which is attached a toxin molecule; 3, a cast off side chain of the first order, antitoxin or antiferment; 4, a toxin or enzyme molecule; *a*, toxophore group; *b*, haptophore group; 5, a toxoid (the toxophore group is destroyed, leaving the haptophore group, *b*, intact); 6, a toxin molecule attached to a cast-off side chain (antitoxin), illustrating the neutralization of toxin by antitoxin in the blood stream. (Kendall.)

with toxin but was also capable of essentially changing the structure of the toxin molecule, it would be necessary to assume that the antitoxin molecule consisted of something more than a simple haptophore group.

Receptors of the Second Order.—These are the agglutinins and precipitins. The nature of agglutinin (precipitinogen) and agglutinin (precipitin) and the manner of their union are symbolized in Fig. 60. The agglutinin (precipitin) molecule is regarded as consisting of a haptophore group, capable of combining specifically with the antigen (a cell or a protein in solution), and of a *zymophore*

group to which is ascribable the agglutinating or precipitating effect. In this respect the agglutinin (precipitin) molecule is similar to the toxin molecule. The toxophore group is analogous to the zymophore group. The zymophore group, like the toxophore group, is more easily destroyed than the haptophore group. When such destruction occurs the agglutinin molecule consists only of a haptophore group, and is then known as an *agglutinoid*. An agglutinoid can satisfy the affinities of its corresponding antigen but cannot bring

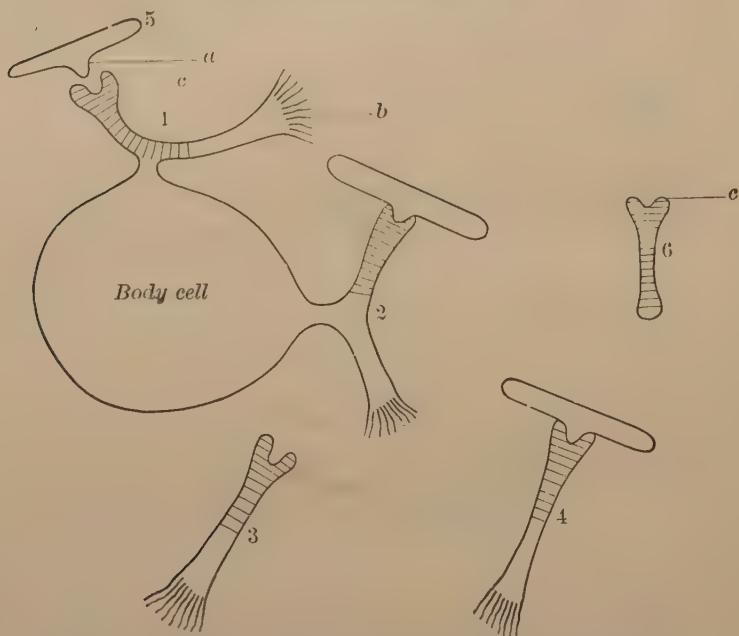


FIG. 60.—Side chains, second order (agglutinins and precipitins). 1, side chain attached to cell; c, haptophore group; b, zymophore group (agglutinophore or precipitinophore group); 2, side chain to which is attached a bacterial cell; a, haptophore group of bacterial cell; 3, a cast-off side chain of the second order (agglutinin or precipitin); 4, a side chain attached to a bacterial cell (agglutination); 5, a bacterial cell; a, haptophore group; 6, an agglutinoid; the zymophore group is destroyed, leaving the haptophore group intact. (Kendall.)

about agglutination. The existence of an agglutinoid-antigen union is inferred from the inability of true agglutinin to combine with or agglutinate antigen which has been saturated with agglutinoid.

Receptors of the Third Order.—These are the bacteriolytic (or more generally, cytolytic) amboceptors. Fig. 61 symbolically illustrates the nature of this antibody and the mechanism of its action. An amboceptor is a molecule consisting essentially of two

haptophore groups. One haptophore group has specific affinities for the corresponding antigen. This might be called an antigenophile group. The other haptophore group establishes the connection with complement or alexin. This is consequently known as the complementophile group. According to this view complement or alexin is the agent which is responsible for the lysis of the cell.

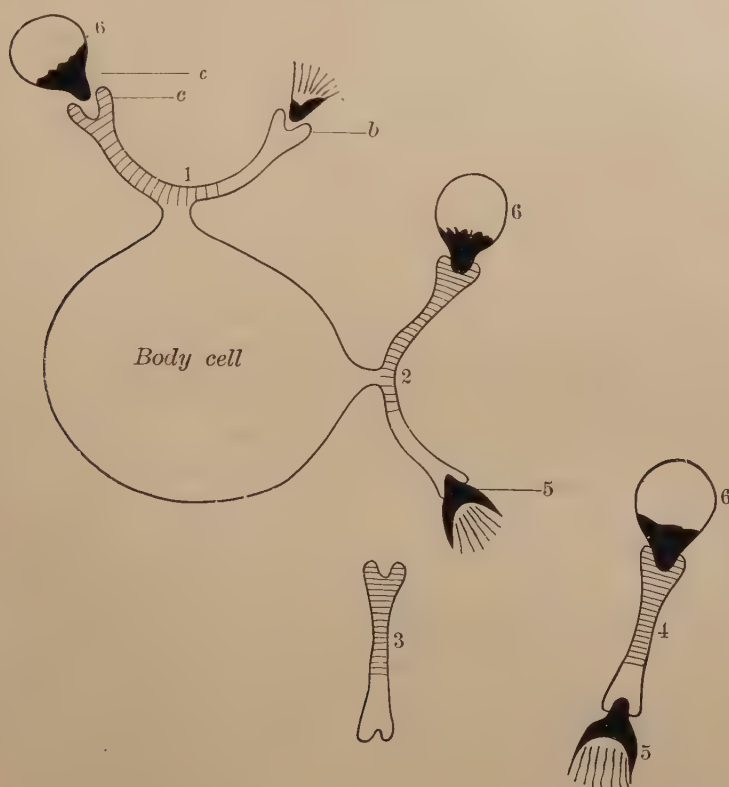


FIG. 61.—Side chains, third order (bacteriolysins, hemolysins and cytolyins). 1, side chain attached to cell; c, haptophore group; b, complementophile group; 2, side chain to which is attached a bacterial cell (6) and complement (5); 3, a cast-off side chain of the third order (amboceptor); 4, a cast-off side chain to which are attached a bacterial cell (6) and complement (5) illustrating lysis; 5, complement. (Kendall.)

Complement may be regarded in a way as comparable to the zymophore group of an agglutinin molecule, which has become detached from its haptophore group. The specificity of cytotoxicity is referable to the antigenophile group of the amboceptor. The function of the amboceptor in cytotoxicity is to establish connection between antigen on the one hand and the lytic agent (complement) on the other.

CHAPTER XIV

PROTECTION AND DEFENSE OF THE HOST AGAINST BACTERIAL INVASION. THE CELLULAR DEFENSES.

It is seen that even according to Ehrlich the constituents of the body fluids, which participate in the defense against infection, are the products of cellular activity. From the earlier studies of Metchnikoff on, the problem of the source of the humoral antibodies has been extensively investigated. The general consensus of opinion today is to look upon the leukocytes, the leukocyte-forming organs, spleen, bone-marrow and lymph nodes, liver and capillary endothelium as the principal producers of antibodies. The statement is also probably true (and not really in conflict with the above view) that the ability to form antibodies is very widely spread and can manifest itself locally wherever antigen is present. For instance, Portis¹ reported that the clasmatoocytes of the rabbit omentum appear to be a definite factor in antibody production following intra-peritoneal injection of antigen. A more general thesis has been developed by Gay and Clark² to the effect that the reticulo-endothelial system of Aschoff (see section on Phagocytosis is very important in the production of antibodies. Departing from the observation and assumption that one colloidal substance when taken up by clasmatoocytes prevents the absorption of a second colloidal substance, they proceeded to saturate the reticulo-endothelial apparatus with trypan blue. When it was thought that this saturation had been accomplished, they injected antigens and observed if any effect was apparent in the production of antibodies. Injections of trypan blue were given to rabbits and rats for about two weeks. Then they were given three injections of sheep erythrocytes on successive days. In rats, the serum of control animals untreated with trypan blue but given similar injections of blood, gave hemolysis in dilutions of 1 to 2500 to 1 to 10,000, whereas in the trypan blue animals it was negative at 1 to 10 in 2 animals and positive at 1 to 160 only in 2 others. The work on the rabbits gave essentially the same result. Similar results also were obtained

¹ Jour. Inf. Dis., 1924, **34**, 159.

² Proc. Soc. Exper. Biol. and Med., 1924, **22**, 1.

in the formation of bacteriolysins and agglutinins to the cholera vibrio. The results on precipitin formation to horse serum after vital staining, however, were variable. If the theoretic basis of these experiments be correct, the conclusion seems necessary that the reticulo-endothelial system does play an important part in the production of antibodies.

An interesting speculation has been offered by Eastwood¹ as a working hypothesis for the study of the mechanism of antibody formation. In all serological work one is struck with the specificity of the reactions, and any satisfactory theory of antibody formation must account for that characteristic. According to the theory suggested by Eastwood this characteristic would be automatically determined. In brief, it is conceived that the antigen may become adsorbed at the surface of the endothelial cells of the capillaries. This phenomenon will alter the "filtering" powers of these cells. The modified transudate or exudate will possess the properties ordinarily assigned to antibodies. There will be a specific relationship between the antigen and these properties because the character of the antigen will determine the nature of the modification undergone by the cells which affects their "filtering" powers. The modification persists for variable times after the disappearance of the antigen because the metabolism of the cells of the capillary endothelium has been altered by the antigen.

It is well known that in no instance does the host rely solely for its protection upon the various antibodies which are demonstrably free in the body humors. In a series of papers² Digby has developed the thesis that the tonsils, Peyer's patches, solitary lymph nodes, and the vermiform appendix should be grouped together as sub-epithelial lymphatic structures continually ingesting the bacteria of the nasal and alimentary channels, with consequent constant immunization of the body against various infections. Another defensive resource is suggested by the work of Scott.³ He found that the resistance of rats to bacterial intoxication (killed streptococci or staphylococci), was greatly decreased by double adrenalectomy and that the condition responsible for the normal resistance was dependent upon the functioning of the adrenal cortex.

The resistance to reinfection with the same parasite, which is seen in many cases and which may persist for many years, cannot

¹ Jour. Hyg., 1924, **22**, 355.

² Guy's Hosp. Gaz., **25**, 203; Lancet, 1912, i, 150; *ibid.*, 1913, i, 1731; Univ. Med. Rec., **3**, 109; China Med. Jour., 1917; also in book form, Immunity in Health, London, 1919.

³ Jour. Exper. Med., 1924, **39**, 457.

be due to the persistence of increased quantities of antibodies in the humors. In the case of typhoid fever, to take an example, the large excess of agglutinins and specific amboceptors demonstrable during the course of the infection rapidly disappears during recovery. The defense during infection and the prolonged resistance many times observable following recovery are certainly due to something more than an excess of free, specific antibodies. The cell is active not only indirectly by the production of antibodies but also directly in varied and in many respects obscure ways. The obvious advantage accruing to the host from the direct ingestion of the parasite by certain types of body cells is discussed below under the topic of *phagocytosis*. It has been suggested, and it is possibly true, that some of the increased resistance offered to an infection following recovery is due to some sort of sensitization of the body cells, enabling them to respond to the stimulus of the same irritant (antigen) more rapidly by the production of an excess of antibodies. It is also true that cellular defense and resistance involve more than the factors mentioned above, *viz.*, phagocytosis and this hypothetical type of sensitization.

As an example may be cited the work of Nukada and Matsuzaki.¹ These authors demonstrated that the tissue cells of the heart of rabbits which had been immunized against typhoid, are altered in such a way that their resisting power is specifically increased against the toxic products of typhoid bacilli. The possible participation of soluble antibodies in this increased resistance was eliminated. The following table shows the difference in resistance exhibited by the hearts of typhoid-immune and normal, control rabbits, when a toxic solution prepared from typhoid bacilli is added to the perfusing fluid.

No. of experiment.	Condition of rabbit.	No. of minutes during which heart continued to beat.
1	{ Immune	50
	{ Normal	20
2	{ Immune	45*
	{ Normal	4
3	{ Immune	20
	{ Normal	4

* Still beating.

This type of cellular defense or resistance is almost certainly of great importance, but next to nothing is known of it to-day. The nature of the intracellular changes is an absolute *terra incognita*.

¹ Jour. Exper. Med., 1924, 40, 661.

PHAGOCYTOSIS AND OPSONINS.

One of the most significant generalizations in the history of pathologic thought was the realization that a physiological analogue could be found for most, if not all, pathological processes. Probably no student can have escaped from a formal course in biology or zoölogy, no matter how elementary, without some recollection of the amœba; a nucleated bit of naked protoplasm spreading itself rather languidly here and there over the microscopic field. In the course of its travels minute particles, bacteria for instance, are encountered and engulfed. A vacuole forms around the particle, which, if digestible, is utilized. This very simple and primitive, nutritive function is retained by at least some of the cells of all animals. When it is encountered in a metazoan cell, it is called *phagocytosis* and the cell itself, a *phagocyte*. This means an "eating cell."

The significance of phagocytosis in infectious disease was first recognized and emphasized by Metchnikoff. The inception of this very important idea (about 1882) is best given in his own words:

"One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defense of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts.

"I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of bloodvessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done.

"There was a small garden to our dwelling, in which we had a few days previously organized a 'Christmas tree' for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star-fish larvæ as transparent as water.

"I was too excited to sleep that night in the expectation of the result of my experiment, and very early the next morning I ascertained that it had fully succeeded."

That experiment formed the basis of the phagocyte theory.¹

This initial experiment started a long train of studies, which are yet far from completion, but which, however, culminated in the publication of a classical work by Metchnikoff.² This presents the evidence for judging of the role of phagocytosis in immunity. He concludes that, "It is clearly proved that phagocytes are susceptible cells which react against morbid agents, whether organized or not. These cells ingest microorganisms and absorb soluble substances. They seize microbes whilst these are still living and capable of exercising their noxious effect and bring them under the action of their cellular contents, which are capable of killing and digesting the microorganisms or of inhibiting their pathogenic action," (p. 543.)

To Metchnikoff, the majority of phagocytes circulated in the lymph and blood. In the vertebrata he recognized two categories of such cells, the macrophages and the microphages, *i. e.*, large and small phagocytic cells respectively. In current terminology, the macrophage is known by several names, *viz.*, the large mononuclear, the large lymphocyte, or the endothelial leukocyte. The genetic relations of these cells, whether they be one or several, is still undetermined. The microphage is the polymorphonuclear leukocyte. Metchnikoff did not distinguish between the three kinds described today, but we know he had reference to the polymorphonuclear neutrophil. The earlier, almost exclusive, emphasis on the leukocytes of the blood may have been determined by the fact that historically the theory of phagocytosis developed in active conflict with the so-called humoral theory of immunity.

Since this time (1902) it has been amply demonstrated that phagocytosis is by no means largely limited to the white cells of the circulating blood. It may be that every metazoan cell with the possible exception of the neuron has preserved this potentiality. It is certain that the phagocytic power is exhibited in high degree by cells of mesenchymal origin whatever be their localization. In this category should be included the various wandering connective tissue cells, the Kupffer cells of the liver, the clasmotocytes of the testis, the cells living in the peritoneal and pleural cavities and the synovial sacs, and finally the entire vascular endothelium. The recognition of the importance of the phagocytic power of these varied types of cells contributed materially to Aschoff's³ decision

¹ Metchnikoff, Olga: Life of Elie Metchnikoff, 1845-1916. London, 1921, p. 117.

² Immunity in Infective Diseases, 1902; English translation, Cambridge Univ. Press, 1905.

³ Ergebn. d. inn. Med. u. Kinderhkl., 1924, vol. 26; Aschoff's Lectures on Pathology, New York, 1924, (Chapter I).

to regard these cells as constituting a functional system. The word "system" is used here as it is used in the terms, cardiovascular or nervous systems. The term in general use for the system, proposed by Aschoff, is the *reticulo-endothelial* system. These facts do not minimize the importance of the macrophages and microphages but do materially supplement our knowledge on this arm of defense and indicate how extensive is the phagocytic reserve against infection.

The practically omnipresent distribution of phagocytic cells is readily demonstrated by a simple procedure.¹ Five cc of a 1 per cent fresh colloidal trypan blue in physiological salt solution are injected intraperitoneally into an albino rat. This is followed by a rapid diffusion of the colloid through the body so that within a few hours a general bluish tint is discernible, first in the ears, snout and paws. In twenty-four hours the rat is distinctly colored blue; a condition which lasts for months. Microscopically visible aggregates of the dye are to be seen in various fixed and wandering cells of mesenchymal origin scattered throughout the body. It is to be noted in this case that the ingested particle was a non-vital, inert substance. Finely divided carbon, as in India ink, and carmin have also been used for a similar purpose. The use of such substances has contributed much to our understanding of certain phases of phagocytosis.

The capillary and sinusoidal networks of the lungs and liver and also of the spleen, lymph nodes and bone-marrow afford an enormous expanse of endothelial surface which possesses the ability of removing particulate foreign matter from the circulating blood. The quantity of endothelial cells in these organs is noteworthy and it has been suggested that these cells are even qualitatively more efficient than endothelial cells elsewhere. Nagao² found that when a suspension of India ink (finely divided carbon) is intravenously injected, the granules are deposited regularly in the endothelial cells of the liver, spleen, and marrow. Similar results were obtained by Wislocki.³ Carbon particles in suspension, when injected intravenously in living rabbits, are deposited in the liver, spleen, lungs, and bone-marrow. In cats a similar distribution was found in the liver, spleen, and lungs, but not in the bone-marrow. The actual phagocytosing of the particles, however, is ascribed by this author to clasmatocytes rather than to the endothelium.

¹ Addison and Thorington: Behavior of the Phagocytic Cells of the Peritoneal Fluid toward Particulate Matter, *Anat. Rec.*, 1918, **14**, 467.

² Fate of India Ink Injected into Blood, *Jour. Infect. Dis.*, 1920, **27**, 527.

³ Fate of Injected Carbon Particles, *Am. Jour. Anat.*, 1924, **32**, 423

It is very easy to verify the observations of foreign particles within the pulmonary or hepatic endothelium. The technic used by Permar¹ is simple and gives good results. Five or 6 cc of a 25 per cent suspension of India ink (1.5 cc Higgin's India ink, black, plus 4.5 cc sterile physiological NaCl solution), is given intravenously to a rabbit. After one or two such injections the rabbit is killed some five or six hours after the second treatment. Various organs are prepared for histological examination. If eosin and methylene blue or hematoxylin be used, it is well to stain rather lightly with the basic dye. The particles of carbon can be easily seen, many of them within the vascular endothelium of the lungs and liver (Fig. 62). When chicken blood is injected intravenously into guinea-pigs, Oeller² found phagocytosis is limited almost exclusively to the endothelial cells of the spleen and liver. In an immunized guinea-pig the corpuscles are hemolyzed in the blood stream and the nuclei are immediately taken up by the endothelial cells of the pulmonary capillaries.

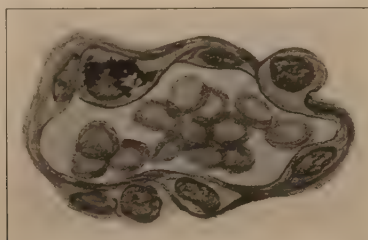


FIG. 62.—A capillary from an alveolar wall in slightly opaque section. One of the endothelial lining cells is greatly enlarged and contains phagocytized carbon granules. Another, similar in every way but devoid of pigment, is seen migrating to the interstitial tissue. (Permar.)

The beautiful work of Drinker and Shaw³ illustrates the phagocytic power of these endothelia. Manganese dioxid in suspension was injected intravenously with precaution to preclude its removal from the blood or deposition in organs through simple capillary blockage. At the end of one hour 90 per cent of the material was recovered in the lungs, liver, and spleen in the following proportions: Lungs, 47 per cent; liver 38.3 per cent; spleen, 4.3 per cent. Histologically it was evident that the vascular endothelium accounted for the retention of these quantities within the respective organs.

¹ Mononuclear Phagocytes in Pneumonia, *Jour. Med. Res.*, 1923, **44**, 27.

² *Deutsch. med. Wchnschr.*, 1923, **49**, 1287.

³ Quantitative Distribution of Particulate Material (Manganese Dioxid), administered Intravenously, *Jour. Exper. Med.*, 1921, **33**, 77.

The conspicuous phagocytic power of the endothelial cells of the lungs and capillaries certainly is of strategic importance in the defense of the body. The intestinal mucosa as we have already pointed out is under various circumstances permeable to various bacteria. The drainage by way of the portal circulation subjects the blood from these parts to the sterilizing effect of the liver before reaching the inferior vena cava. In a similar fashion the venous blood from the right heart, representing the drainage of the body, is subjected to the sterilizing effect of the lungs before being distributed to all parts of the body by means of the larger circulation.

The experimental work described above relates only to the ingestion of inert, non-living foreign particles. Although the data obtained in this way is not *per se* proof that living bacteria are subjected to the same fate, *i. e.*, phagocytosis by mesenchymal cells other than the circulating leukocytes, there is no lack of evidence that this does occur. Borel¹ has described the phagocytosis of tubercle bacilli by endothelial cells. The same thing has been observed in the case of the anthrax bacillus by Werigo² and by Lemaire.³ Adami⁴ has observed the phagocytosis of colon bacilli, experimentally injected intravenously in the rabbit, by the endothelium of the liver sinusoids. Within these cells the bacteria apparently underwent a type of disintegration which reminded one strongly of the extracellular Pfeiffer phenomenon. The natural immunity of the pigeon to the pneumococcus has been ascribed by Kyes⁵ to the removal of the microorganisms by the Kupffer cells of the liver. Hopkins and Parker⁶ found that the rapid removal of streptococci injected intravenously into rabbits and cats was accomplished by the endothelial cells of the lungs, liver, and spleen.

OPSONINS OR BACTERIOTROPINS.

As the theory of immunity by phagocytosis was developed by Metchnikoff and his school, it was regarded by many as in conflict with another theory of immunity, the humoral, which was based on the protective substances in solution in the body fluids. It was soon found out, however, that these two views were not incompatible but rather must be combined at least in some instances if one

¹ Ann. de l'Inst. Pasteur, 1893, **7**, 593.

² Ibid., 1894, **8**, 1.

³ Arch. de méd. expér., 1899, **11**, 556.

⁴ Jour. Am. Med. Assn., 1899, **33**, 1509.

⁵ Jour. Infect. Dis., 1916, **18**, 272.

⁶ On authority of Zinsser: Infection and Resistance, 3d ed., 1923, p. 341.

were to have a somewhat clearer conception of the truth. The means for this compromise was opened by the discovery that phagocytosis was more marked when it occurred in the presence of the serum of an individual, convalescent or recovered from or intentionally actively immunized against the microörganism in question, than when the serum of a normal individual was used. There are three conceivable explanations for this fact: (1) The immune serum contains something (or is possessed of a property) which stimulates the leukocyte to greater efforts; (2) the immune serum acts upon the bacterium, rendering its ingestion easier, and (3) which is merely the formal combination of (1) and (2). It was soon found (Wright) that the second explanation was the correct one. An outline of the means of demonstrating this follows:

I. (a) A suspension of leukocytes is left in contact with the immune serum for a given length of time.

(b) The leukocytes are washed free of the serum.

(c) These washed leukocytes are added to the suspension of bacteria in question and incubated for a given length of time.

(d) Smears are made from this mixture, stained and (1) the number of bacteria per leukocyte or (2) the number of phagocytes per 100 leukocytes estimated. Let X represent this number.

II. (a) The suspension of bacteria is left in contact with its homologous immune serum for a given length of time.

(b) The bacteria are washed free of the serum.

(c) The washed bacteria are added to washed leukocytes and incubated for a given length of time.

(d) Same as above under I. Let Y represent this number.

If X were greater than Y it would mean that the serum increased phagocytosis by acting upon the leukocytes; but it is always found that Y is greater than X, and appreciably greater, which indicates that the serum acts by modifying the bacterial cell. The validity of the third conceivable explanation may be tested in this way.

III. (a) The leukocytes are subjected to the action of the immune serum and then washed free from it.

(b) The bacteria are treated in the same way.

(c) The washed leukocytes and washed bacteria are added together and incubated.

(d) Same as in I. Let Z represent this number.

The value Z never significantly varies from Y, thus showing that no additional advantage comes from treating the leukocytes with the immune serum and that the action of the serum on the bacteria alone is adequate to account for the increased phagocytosis.

The term *opsonin* (signifying "I prepare a feast for") is given to this substance in, or property of, the immune serum which is fixed by the homologous bacteria, altering them in some way so they are more readily ingested by the leukocytes. Opsonins exhibit the characteristic of specificity, so common among the various antibodies. Their chemical composition and structure are unknown. On the basis of differences in the readiness with which these substances are injured by heat, Neufeld has distinguished between *opsonins* (normal opsonins) and *bacterio-tropins* (immune opsonins). The former group is thermolabile, being inactivated by 56° C. for one hour; the latter (bacteriotropins) is not inactivated under these conditions. The "opsonins" which are found in far larger quantities in the serum of convalescent or immunized individuals, fall in the category of the bacteriotropins.

The content of a patient's serum in opsonins or bacteriotropins has been used for the diagnosis of infection. If the individual exhibit a content of these antibodies remarkably above normal, it is regarded as strong evidence that at least one factor in the infection is the type of bacterium used in the determination. If the content be remarkably low, this is regarded as evidence either that the patient is infected with the organism in question but is offering a weak defense or that the patient, if not infected, is abnormally susceptible to infection with that organism. The test devised to determine the opsonic content may be spoken of as the opsonic test and its result is stated as an "opsonic index." Two (I and II) mixtures are made as follows:

I. (a) A unit volume of a suspension of leukocytes, usually collected from a healthy individual. These cells have been collected in a citrate solution to prevent coagulation, and have been subsequently washed free of blood plasma and citrate. When used they are suspended in physiological (0.85 per cent) sodium chlorid solution.

(b) A unit volume of a homogeneous suspension of the bacteria in question. The density of this suspension is of importance, but it is difficult to give general rules on this point. Further, this suspension should be as free from clumps as possible.

(c) A unit volume of the patient's blood serum which has separated from clotted blood.

II. *a* and *b* as above in Mixture I.

(c) A unit volume of the mixed sera of a several, presumably normal individuals.

These mixtures are incubated for from fifteen to thirty minutes,

when smears are made on clean slides and appropriately stained. The number of bacteria found on microscopical examination within at least 100 leukocytes is counted for each mixture separately, and the average number per leukocyte is reached. Such an average is often spoken of as the *phagocytic index*. The average number for Mixture I (patient's serum) is divided by the average number for Mixture II (pool of normal sera) and the quotient expressed decimally is the *opsonic index*.

To illustrate: If the average number of ingested bacteria per leukocyte in the preparation from Mixture I were 8, and in the case of Mixture II were 12, the opsonic index would be $\frac{8}{12} = 0.66+$.

It is, as a matter of fact, difficult to be sure that one's count of the number of bacteria within a leukocyte represents a close approximation to reality. If one be critical with one's own sense experiences, much uncertainty on this point is felt. It is certainly much easier to decide whether a particular leukocyte contains or does not contain bacteria, irrespective of their numbers. As a consequence this criterion, the frequency of bacteria-containing leukocytes, is sometimes substituted for the classical procedure of Wright given above, as a method of estimating the relative quantity of opsonins in sera. The technic, including the staining of the smears is the same. In the slides from Mixtures I and II a large number of leukocytes, 200 or 300 at least, is counted, noting how many of them are phagocytic, *i. e.*, contain bacteria. From this the percentage of phagocytes in each mixture is calculated. The percentage of phagocytes in Mixture I (patient's serum) is divided by the percentage of phagocytes in Mixture II (pool of presumably normal sera) and the quotient, expressed decimally, gives an opsonic index.

It must not be forgotten that opsonic indices obtained by this latter method of calculation are *not* comparable with those obtained on the basis of the average number of bacteria per leukocyte.

Another, and very satisfactory, method of determining the relative opsonin content of sera is to make a number of mixtures of leukocytes, bacteria and serum, using various *dilutions* of the serum. The dilution of the patient's serum which gives no higher degree of phagocytosis than that obtained when physiological salt solution is substituted for the serum is selected for comparison with a similar dilution observed in the mixtures containing the pooled sera of normal individuals.

In any consideration of phagocytosis we encounter three factors:

(1) The leukocyte; (2) the serum, and (3) the bacteria. We have already seen that in respect to their power of facilitating phagocytosis, various sera show marked differences. In respect to the bacterial factor, it suffices to note that virulent bacteria resist phagocytosis better than avirulent strains of the same species. The possibility that leukocytes from different individuals may vary in respect to their ingesting powers, is usually ignored. As a matter of fact, however, differences do exist both in health and disease.¹ Recently Martley² has offered evidence that leukocytes, from an individual belonging to Blood-group II (Moss), possess a phagocytic power some 25 per cent higher than do leukocytes from an individual belonging to Blood-group IV.

The determination of the opsonic index has been used for three clinical purposes:

1. Diagnosis—reference has already been made to this. It should be noted that infections with the pyogenic organisms usually show a low index, *i. e.*, below unity. In the section on Vaccins it will be noted that the earlier workers used the opsonic index as an aid in selecting the bacteria to be included in an autogenous vaccin.

2. The determination of the potency of sera for passive immunization.

3. In the treatment of a case, especially in bacteriotherapy, as a clinical index of the time to administer the vaccin and as an index of the progress of the case, whether favorable or unfavorable.

This third clinical purpose to which the opsonic index has been put requires some elaboration. When a vaccin is administered, the opsonin content of the serum is at first diminished. This is usually transient and is followed by a rise, which usually reaches a value slightly greater than the value before the injection. The fall in the opsonic index is spoken of as the "negative phase" and the subsequent rise, as the "positive phase." It was found that the injection of a second dose of the vaccin during the negative phase was followed by a further drop and was, therefore, contra-indicated. The opportune time for the administration of the vaccin was regarded as during the positive phase. The accompanying figures illustrate the body response to vaccin treatment in terms of the opsonic index.

¹ Park and Biggs: Jour. Med. Res., 1907, vol. 17. Glynn and Cox: Jour. Path. and Bacteriol., 1910, vol. 14. Hektoen: Jour. Am. Med. Assn., 1911, vol. 57. Rosenow: Jour. Infect. Dis., 1910, vol. 7. Tunnicliff: Jour. Infect. Dis., 1911, vol. 8.

² Comparative Phagocytic Properties of the Leukocytes, Lancet, 1924, 206, 126.

The opsonic test of Wright and its modifications have not proved applicable as *routine* clinical methods for quantitatively measuring

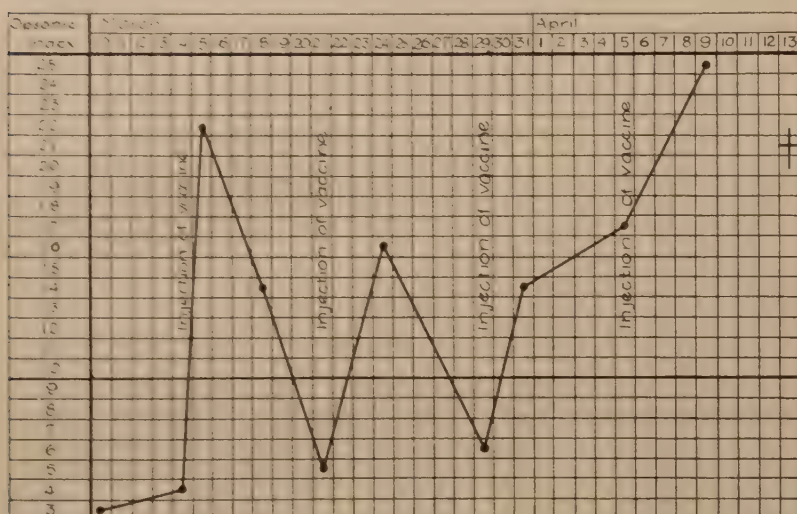


FIG. 63.—Opsonic curve in a case of noma injected with killed culture of fusiform bacilli and spirochetes. March 15, growth on one blood-agar slant injected subcutaneously; March 21, growth on one and a half blood-agar slants injected subcutaneously; March 29, growth on two blood-agar slants injected subcutaneously; April 5, growth on one and a half blood-agar slants injected subcutaneously. (Tunncliffe.)

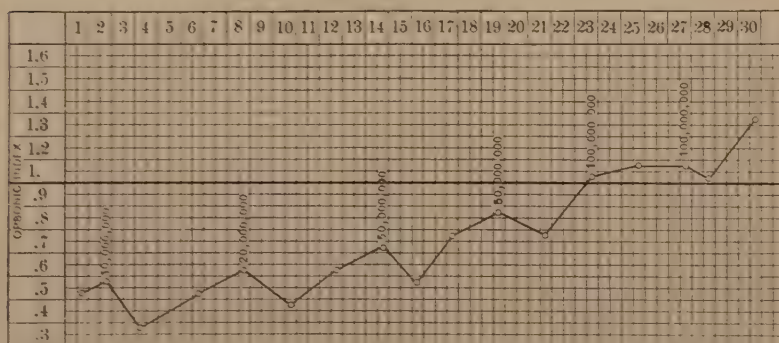


FIG. 64.—Chart showing effect of inoculation in a case of streptococcus infection of the nasal sinus. Note negative and positive phases following the injection of vaccine beginning with 10,000,000 and increasing to 100,000,000. Circles indicate the index. (Kirk.)

the phagocytic defense. The technic is exacting and time-consuming. The multiplicity of factors entering in the reaction requires that; and one of the most important and uncontrollable of these

factors is the technician himself. The error due to the "personal equation" is likely to be high. The values obtained by one individual at various times may be comparable, but it would be rash to compare the particular values obtained by one observer with those obtained by another observer. At the present time the determination of the opsonic index is rarely done except in connection with research problems. This does not mean that phagocytosis is relatively unimportant or that opsonins are negligible antibodies; it only means that the methods available for accurately measuring these phases of defense leave much to be desired in the way of reliability and simplification.

The Mechanism of Phagocytosis.—In this connection we have already considered the importance of certain constituents of the serum; but apart from specific antibodies, as opsonins, the serum seems to play an important role. Fenn¹ observed even in the case of carbon particles that there was almost no phagocytosis in the absence of serum or if the serum had previously been heated.

Addison and Thorington² would emphasize that the size of the molecular aggregate is an important factor in determining not only the rapidity and degree of phagocytic response to inert particular matter, but also determines in large part the type of phagocyte predominating in the reaction. An examination of Fenn's reports gives facts in consonance with this view. He observed differences in the degree to which particles of quartz or carbon were ingested, even when their size-differences did not exceed the limit of 1μ to 2μ .

It must not be forgotten that data obtained with the use of inert, non-living, foreign particles are not immediately applicable to problems dealing with the living bacterial cell. This is apparent from one of Fenn's studies.³ He established that the phagocytosis of solid inert particles (quartz and carbon) follows the law for a monomolecular reaction, *viz.*:

$$K = \frac{1}{t} \cdot \log \frac{A}{A-x}$$

K = rate of phagocytosis.

A = number of particles originally present.

x = number of particles ingested by leukocytes in time, t .

K usually proves to be a constant, meaning that the same per cent of the particles present is being ingested per unit of time. In other words, it is *not the number of collisions* (between leukocyte

¹ Jour. Gen. Physiol., 1921, 3, 465.

² Loc. cit.

³ The Phagocytosis of Solid Particles: II. Carbon, Jour. Gen. Physiol., 1921, 3, 465.

and particle^o) but the chances of collision which determine *K*. When we turn to the phagocytosis of bacteria, however, the available evidence indicates that the law for a monomolecular reaction does not apply - possibly because of the toxic effect upon the leukocytes of bacterial products.

A factor in phagocytosis which is operative in the case of bacteria and other living foreign cells but is not operative in the case of such inert particles as carmin, carbon or quartz, is the attractive or repellent influence upon the leukocyte of substances diffusing from the invading cell. Any living, free-moving cell, as the leukocyte, may be attracted or repelled by certain chemicals in solution. This is known as chemotaxis or chemotropism. The attraction of the cell is the phenomenon of *positive* chemotropism or prochemotropism; the repulsion of the cell is the phenomenon of *negative* chemotropism or apochemotropism. One and the same substance may be positively or negatively chemotropic according to its concentration or other conditions. Likewise one and the same substance may be positively chemotropic for one type of cell and negatively chemotropic for another type of cell.

It has been abundantly proven that bacterial products act chemotropically upon leukocytes. This action may be positive or negative. The clearest evidence of positive chemotropism is offered by the emigration of leukocytes from the bloodvessels and their accumulation around the invading bacteria. This is the characteristic picture of localized pyogenic infections and is a prominent feature of the inflammatory reaction. Positively chemotropic influences also certainly play a part in the hyperleukocytosis of infections. The bacterial substances, acting prochemotropically have already been discussed under the heading of the bacterial proteins of Buchner. It must not be forgotten that the disintegrating products of necrotic tissues also exert a strong, positively chemotropic action on leukocytes. This we see in abscesses, acting in conjunction with the bacterial constituents, disintegration products, and metabolites.

Unfortunately, we know little of negative chemotropism in infections. It may be a factor in some leukopenias, as for example occur as anaphylactic manifestations.

The phenomena of chemotropism and of the other tropisms, as they are manifested by amœboid cells, are usually and very satisfactorily interpreted in terms of changes in surface tension. If the chemical reduces the surface tension, the cell moves toward the point from which the chemical is diffusing; if the surface tension be increased, the opposite effect, that of repulsion, is obtained. The

actual engulfing of the bacterial cell is likely a continuation of the same process and is determined by changes of surface tension in the leukocyte.

The destruction of the ingested bacterium is usually ascribed to the various intracellular enzymes which have been identified in the leukocyte. Sometimes the virulency or the large number of the bacteria within the phagocyte kills the latter. This may occur at the site of invasion or later after the body cell has migrated to some other part of the system. In this way infection may be spread. In the history of the development of the concept of phagocytosis this was regarded by some pathologists as the ordinary and chief result of phagocytosis. It is still admitted that dissemination of bacteria through the body may occasionally occur in this way.

Phagocytosis in Oral Infections.—Polymorphonuclear neutrophils are constantly even in normal mouths passing through the epithelium of the oral mucosa onto the free surface. Such cells, which are also found in saliva as "salivary corpuscles," are potentially phagocytic and may contribute in part to the diminution of the flora of the mouth.

The acute inflammations of the periodontal and periapical tissues are usually occasioned by the pyogenic bacteria, and the resulting exudates always are rich in phagocytic cells.

The relation of vaccins to oral diseases is considered in detail elsewhere. At this place we need note no more than that Wright (to whom the introduction of these agencies is largely due), considered that they were chiefly operative by increasing the opsonin content of the blood plasma.

The use of dichloramin-T in oral infection is fairly general. This drug when applied attracts large numbers of leukocytes and the phagocytosis thereby facilitated not unlikely plays some part in the resulting beneficial results. In this connection should be mentioned the paper of Chappel.¹ He recognized that most of the acute oral infections are of the pyogenic type in which polymorphonuclears largely participate in the defense. He also pointed out that the present tendency in rational therapeutics is to be content with a modest reënforcement or direction of natural processes. With these thoughts in mind, the inference is easy that "we must study to apply those remedies (in the cases noted above) which will aid in promoting phagocytosis instead of hindering it." There seems to be a trend away from the use of strong antiseptics or germicides

¹ Jour. Nat. Dent. Assn., 1917, 4, 889.

in dentistry, which is encouraging because these substances are either leukocidal or negatively chemotropic for leukocytes.

Mendel¹ believes that the phagocytic behavior of the polymorphonuclears from the gingival trough is clinically significant. According to this author, in young normal individuals there are 5 to 6 phagocytes per 100 leukocytes. With the appearance of a hyperleukocytosis the phagocytic ratio rises to a mean of 50 to 60 and up even to 80 per 100; but in well-marked pyorrhea alveolaris the ratio is rather low, *e. g.*, 9, 10, 15, 16, 18, 23 per 100 leukocytes. If the results of these observations were established as generally valid, the simple microscopical examination of the gingival exudate would prove to be of high clinical value. It would permit the recognition of a prepyorrhetic state. An hyperleukocytosis or a phagocytic ratio ranging approximately from 50 to 60 or more, would indicate incipient and even microscopically unrecognized periodontal disease.

In the examination of large numbers of oral smears from young unselected individuals it was found that a larger number of polymorphonuclear leukocytes, than an isolated one here and there, is invariably pathognomonic of gingival irritation. The existence of such lesions in most instances was unknown to the patient and even unrecognized until after attention had been particularly called to them by the microscopical examination. Then it was found that the site, from which the material had been collected for the smear, was subjected to the trauma of salivary calculus, of a poorly adapted band or crown, of an unfinished gingival margin of a filling, or of abnormal occlusal relations. The point emphasized by these observations is that minute and clinically inconspicuous lesions were recognized easily by simple microscopical demonstration of phagocytic cells, and that such lesions are widely regarded as being initial stages in refractory processes, often terminating in the exfoliation of the teeth.

¹ Ann. di odontol., 1917, 2, 103.

CHAPTER XV.

RESISTANCE AND IMMUNITY.

NATURAL IMMUNITY.

It has long been known that different species, races, families and individuals are not equally susceptible to the same infections. We know, to give particular examples, that typhoid, cholera, syphilis, and influenza are spontaneously or naturally contracted only by human beings. We know that Algerian sheep are less susceptible to anthrax than are the common ovine races of Europe. The presence or absence of susceptibility to diphtheria or to scarlet fever among different families or individuals has been put on a scientifically determinable basis by the Schick and Dick tests respectively. We know that in every epidemic certain individuals inexplicably remain untouched. These are instances of so-called *natural* resistance or immunity.

The factors which account for natural immunity, are far from being known in full; to say nothing of their evaluation. At the present time a fundamental, systematic discussion of this subject seems impossible. A few more or less obvious instances and illustrations follow:

Probably the first factor to which attention was directed as contributing to the presence of natural immunity, was the normal body temperature of the macroörganism in comparison to the optimum temperature of the microörganism. In a classical experiment Pasteur showed that the normal refractoriness of hens to anthrax could be abolished by the simple procedure of artificially lowering the body temperature of these birds. This was accomplished by tying them in a stream of cold water which flowed well up on their bodies. A similar observation has been more recently made. Pigeons are normally resistant to infection with the pneumococcus, but become susceptible when their body temperature is deliberately lowered either by the administration of antipyretics¹ or by feeding them on a diet lacking in the antineuritic vitamin.

Another possible factor which may be operative in some cases of

¹ Strouse: Jour. Exper. Med., 1909, 11, 745.

natural immunity, is that the cells of the macroörganism are wanting in affinities for any of the constituents or products of the micro-organism in question; or perhaps if such affinities exist, the substance derived from the microörganism does not exert a toxic action. This view has been advanced for the complete innocuousness of diphtheria or tetanus toxin toward the lizard. Tetanus toxin injected into a susceptible animal like the guinea-pig rapidly disappears from the body fluids (bound by the susceptible cells) and death results. In the lizard the toxin remains free and unbound for months in the body fluids, and the animal apparently suffers no untoward effects. It is further incapable of producing antitoxin.

Likewise doves, inoculated with tetanus spores and with large amounts of tetanus toxin circulating in their blood, show no signs of disease. The nervous tissue of doves, unlike that of susceptible mammals, is unable to combine with this toxin and remove it from solution. This fact, indicating an absence of affinities between the tetanus toxin and the nerve cells of doves, is regarded as an important factor in the natural immunity of these birds to this kind of infection.

Other instances of natural immunity appear to be due to a passive transfer of immune antibodies from an immune mother to the fetus *in utero* or to the nursing child through the secretions of the maternal mammary glands. This mechanism is suggested by the percentages of individuals immune to diphtheria according to the Schick test at different ages.

Age.	Per cent immune to diphtheria.
1 to 6 months	85
7 to 12 "	50
1 to 2 years	45
2 to 4 "	35
4 to 6 "	60
6 to 8 "	65
8 to 20 "	75
20 to 40 "	82
Over 40 "	88

The high percentage of resistant individuals for the first few months after birth may be due to the antitoxin which has been passively acquired *in utero* from immune mothers. The passive transfer of immunity to toxins was early demonstrated by Ehrlich in the case of guinea-pigs. The offspring of immune mothers were also immune at birth. This is not to be confused with a phenomenon of genuine heredity. Paternal immunity had no effect in rendering the offspring immune.¹ The agglutinin for the typhoid

¹ Ehrlich: Ztschr. f. Hyg., 1892, 12, 183.

bacillus has been found by Learmouth¹ in the serum of the offspring of immunized female guinea-pigs. The agglutinin was present before the ingestion of the colostrum. The obvious inference is that the agglutinin as such may pass through the placenta. It is interesting that the quantity of agglutinin in the fetal serum may equal or even exceed that in the maternal serum. Burhaus and Gerstenberger² have shown that in 30 per cent of the series examined by them the serum from the umbilical cord, *i. e.*, from the fetal circulation, showed a protective power against the three fixed types of pneumococci. In general, however, successful demonstration of the transmission of antibodies through the placenta to the fetus has been very rare.³ Another possibility to be considered is that the antigen itself has passed through the placenta and induced a condition of active immunity in the fetus.

It has been demonstrated that the ingestion of colostrum increases the resistance of calves to certain infections. Agglutinins were demonstrable in this liquid and also in the serum of the young animal after, but not before, its ingestion.

The blood, or its serum, of naturally immune animals exerts in some instances an inhibitory influence upon bacterial growth or even a true bactericidal effect. Flexner⁴ noted that the serum of the rat (immune) is destructive to the anthrax bacillus while that of man and mouse (both susceptible) has little or no effect upon this microorganism. More recently Heist and Solis-Cohen⁵ have reported that the whole, coagulable blood of animals naturally resistant to infection by a given microorganism possesses bactericidal or bacteriostatic power against that organism. Such power is lacking in the blood of animals naturally susceptible to infection with that organism. For example, pneumococci seeded *in vitro* in the fresh, whole, coagulable blood of the mouse (susceptible), multiply with great rapidity. When similarly seeded in the blood of the naturally immune chicken, pigeon, cat or dog, the pneumococci disappear. Diphtheria bacilli grow in the blood of the guinea-pig (susceptible) but disappear in the blood of the rat (naturally immune). Meningococci multiply in the blood of the mouse (susceptible) but are destroyed in the blood of the relatively immune rabbit. The globoid bodies of poliomyelitis grow in human blood but succumb when inoculated into rabbit's blood.

¹ Jour. Hyg., 1923, **22**, 100.

² Am. Jour. Dis. Child., 1924, **28**, 416.

³ Compare Musselman (Am. Jour. Obst. and Gynec., 1924, **8**, 141).

⁴ Jour. Exper. Med., 1896, **1**, 576.

⁵ Jour. Am. Med. Assn., 1924, **83**, 824.

Another factor in the great natural resistance of some species to some infections is the ability of certain cells of these species to ingest and destroy intracellularly the microorganisms in question (phagocytosis). In the pigeon which is naturally immune to pneumococcal infection, pneumococci are removed from the circulation by the phagocytic action of the Kupffer cells of the liver.¹ Twenty-five hundred million living tubercle bacilli can be injected intraperitoneally into the rat without the appearance of any symptoms or signs of disease. The degree of this natural resistance is obvious when compared with the fact that 20 to 30 tubercle bacilli (bovine) introduced subcutaneously will almost invariably result in a fatal infection in the rabbit. In the guinea-pig even a smaller number is effective. Gloyne and Page² pointed out that the serum of the rat is without bactericidal action on the tubercle bacillus *in vitro* and that repeated injection of the rat serum afforded no protection to guinea-pigs injected with tubercle bacilli. On the other hand, phagocytosis of these microorganisms in the rat is very efficient and was regarded by these authors as constituting the most marked part of the mechanism of natural resistance in this case.

The probable importance of phagocytosis in natural immunity appears from the interesting work of Metalnikov.³ He found that the larvæ of the insect, *Galleria mellonella*, possess an extraordinary resistance to massive injections of bacteria; but in the majority of instances it was impossible to demonstrate the presence of antibodies. The antimicrobial defense seemed to be almost exclusively dependent upon the phagocytic action of certain cells of the larvæ.

The high natural resistance of the frog to *Staphylococcus aureus* has been studied by Pickoff.⁴ The low body temperature does not appear to be a factor because it was found possible to warm frogs to 34° C. without causing infection. Bactericidal power of the body fluids is lacking. These fluids are good culture media *in vitro* and *in vivo*. Positive blood cultures have been obtained as late as fourteen days after the injection of the bacteria. When anatomical conditions permitted a localization of the bacteria, the leukocytes formed an effective local mechanism of defense both by phagocytosis and by the mechanical blocking of the region involved. Most of such organisms as did reach the blood stream, were ingested and destroyed by Kupffer cells of the liver. Thus again in this case phagocytosis seems to be an important factor in the natural immunity.

¹ Kyes: Jour. Infect. Dis., 1916, **18**, 272.

² Jour. Path. and Bacteriol., 1923, **26**, 224.

³ Ann. de l'Inst. Pasteur, 1923, **37**, 528.

⁴ Jour. Infect. Dis., 1923, **32**, 232.

ACQUIRED IMMUNITY.

An animal which is naturally susceptible to infection with a given microorganism can acquire a high degree of resistance to the same infection. This may happen accidentally, as when an individual spontaneously contracts, for example, smallpox or diphtheria or scarlet fever or typhoid. The fact that recovery from such a disease often left the individual far less likely to contract the same disease again, was observed so early that it is not known when or by whom attention was first called to it. In preceding sections some of the factors (antibody formation and phagocytosis) contributing to the recovery of the patient, have been considered. The increased quantity of antibodies cannot, however, account for the prolonged refractory state following recovery. For example, the agglutinin content of the serum of a typhoid convalescent soon reaches a value far too low to explain the high resistance to typhoid infection manifested by that individual for years to come. In brief, the factors responsible for the immunity acquired as a result of successfully surviving a natural infection, lie still in the unknown.

The recognition of the fact that recovery from a spontaneous infection left that individual distinctly protected against future attacks, however, at an early date suggested to imaginative minds the desirability of deliberately and artificially inducing the same resistant condition. This idea apparently occurred independently at several times and in geographically well separated cultures. As far as current Western European civilization is concerned, the development of this idea was initiated by the remarkable Lady Mary Wortley Montague. At Constantinople she had observed that individuals whom it was desired to protect against the full ravages of smallpox, were when young and in good health inoculated with the material from the pustule of an actual case of smallpox. The disease usually followed but would habitually run a mild course. It is interesting to those who enjoy the economic interpretation of history, that the application of this measure was chiefly limited to children who were destined by their parents to be sold to the harems.

As a result of the efforts of Lady Mary Wortley Montague after her return to England the practice of deliberate inoculation with smallpox to protect against smallpox was to some extent taken up. The next step, which removed practically all danger from the artificial immunization, was made by Jenner. The substitution of material from the lesion of cowpox for the material from the pustule

of actual smallpox made the process much safer to the individual without detracting from its efficacy. The extension of intentional, deliberate, artificial immunization in human medicine will be more fully considered at the end of Part II.

The next step in the development of this idea came in the early '90's of the nineteenth century. The injection of toxin, such as that from the diphtheria or tetanus bacillus, into a susceptible animal resulted in the formation by the cells of that animal of a specifically neutralizing antidote, the antitoxin. A high antitoxin content in the blood plasma protected the animal against quantities of toxin which would kill many animals which had not previously been treated with toxin injections. The thought occurred: Why cannot the antitoxin-rich serum of one animal be artificially introduced into a second animal in order to protect this second animal against what otherwise would be a disease-producing or even fatal dose of the corresponding toxin? Experiment showed that this could be done. Increased resistance could be conferred upon a susceptible individual of one species by the injection of the serum of an immunized individual of the same or of a different species.

Active and Passive Immunity.—Where the resistant state is acquired as the result of the activity of the cells of an individual in response to the presence of an antigen, one speaks of *active* immunity, or *actively acquired immunity*. The antigen may have gained access to the individual accidentally in the course of a natural infection. On the other hand, where the resistant state is acquired indirectly by the deliberate injection of antibodies, manufactured by the cellular activity of another individual, one speaks of *passive* immunity, or *passively acquired immunity*. This condition is always artificially induced by conscious human endeavor, except in such cases where the antibodies are manufactured by the cells of the maternal organism and are secondarily passively conferred upon the fetus by passage through the placenta.

There is a very real and clear difference between active and passive, acquired immunity. Wherever the immunity results as a response to the actual presence of the antigen, we have active immunity. Consequently the immunity acquired following all types of vaccination is an active immunity. It is due in at least most instances to the operation of several factors, only one of which is the formation of antibodies. Active immunity is more slowly acquired than is passive immunity, but when once acquired persists much longer. Passive immunity in an individual is due to antibodies which were produced in another individual, actively immu-

nized. The cells and tissues of the first individual have done nothing in the production of these antibodies. The first individual is merely the beneficiary, the *passive* recipient, of the results of the activity of another individual. Passive immunity is probably solely attributable to antibodies. When artificially induced, immune sera (sera of naturally or artificially, actively immunized animals) or the derivatives of immune sera are always used. The sera must be introduced parenterally. The state of passive immunity appears as soon as the injected serum has been absorbed from the site of injection and found general distribution. When injected intravenously, passive immunity is almost immediately acquired. A higher degree of resistance in a shorter period of time can be secured by passive immunization than by active immunization. The condition of passive immunity, however, does not last as long as that of active immunity (Fig. 65).

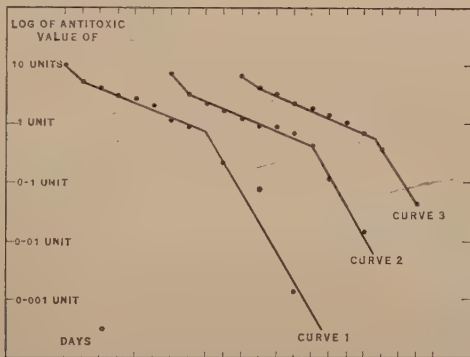


FIG. 65.—Showing the antitoxic value of the blood of three normal rabbits at different intervals of time after intravenous injection of 0.5 cc of unconcentrated horse serum containing 750 units of diphtheria antitoxin. Antitoxic value of units per cubic centimeter. (Glenny and Hopkins: *Jour. Hyg.*, November, 1922.)

The sera that are used to confer passive immunity are described as (a) antitoxic and (b) antibacterial. These agents are employed prophylactically and therapeutically. The antitoxic sera against diphtheria and tetanus are highly effective. Potent sera can also be produced against the toxins of *Clostridium botulinum* and of the anaërobic bacteria of gas gangrene. The evidence is rapidly accumulating that the serum treatment against scarlet fever will prove of the greatest clinical value. Presumably the immunity conferred is antitoxic in type. Antibacterial sera have proved less satisfactory than antitoxic sera in human medicine. The diseases in which

some encouragement has been received for serum treatment, are epidemic meningitis and pneumococcal (Type I) pneumonia. In these cases the immunity is probably not antitoxic.

Local and General Immunity.—Just as it is possible to speak of local and general infection it is possible to speak of local and general resistance or immunity. Most studies in the past have emphasized the problems of general immunity. Certain facts, however, stand out that make it unwise to ignore the condition designated as local immunity. Active immunity, either naturally or artificially acquired, may be either local or general. A very common instance of local immunity is afforded by epidemic parotitis or mumps. When the infection is unilateral instead of bilateral, a second attack will usually involve the side which had not been previously affected. Roemer dropped a solution of abrin (comparable to a bacterial toxin) into one eye of a rabbit. This eye acquired immunity while the other eye did not. Besredka¹ has put a great deal of stress on local immunity. For example, it is possible to vaccinate a rabbit *per os* by means of heated cultures of Shiga's dysentery bacillus. The sequent resistance is not due to the formation of humoral antibodies. Besredka regards this case of immunity as an *entero-immunity*, resident in the parenchyma cells of the intestine, which originally had been susceptible. Another instance of local immunity is that which occurs in the case of anthrax following *intracutaneous* vaccination (Besredka, Plotz, and others. Delater² and Besredka³ have with apparent clinical success applied streptococcal and staphylococcal vaccins or sterile filtrates of cultures of these bacteria as dressings locally upon the area it was desired to immunize. The Goldenberg vaccin (*q.v.*) which was developed for the treatment of periodontal infections, aims to increase the resistance of the gingivæ locally.

¹ Paris méd., 1922, **43**, 460.

² Presse méd., 1924, **32**, 3.

³ Ann. de l'Inst. Pasteur, 1924, **38**, 565.

CHAPTER XVI.

RESISTANCE AND IMMUNITY. ARTIFICIAL ACTIVE IMMUNIZATION.

THE word *vaccin* is derived from the Latin word *vacca*, meaning *cow*. It referred originally to the material from cowpox (*vaccina*) employed by Jenner to protect human beings against smallpox (*variola*). Its meaning has since been so widened that it includes any microörganismal constituent or product which is used with the deliberate intention of inducing specific, active immunity, either prophylactically or therapeutically. For this purpose reliance has been placed upon the injection:

- (a) Of attenuated organisms.
- (b) Of fully virulent organisms in non-lethal doses, or into a resistant locus.
- (c) Of toxins.
- (d) Of extracts of bacterial cells.
- (e) Of killed organisms.

(a) Various methods have been used to attenuate (lessen the pathogenicity of) organisms. Animal passage may enhance or diminish the virulence. Although the virus of smallpox is unknown, it is probable that antivariolic vaccination is practicable because of attenuation from passage through the cow. It had long been a popular belief that milk-maids who had suffered from a cutaneous eruption, contracted from an affected animal (cowpox) were immune to smallpox. Jenner, an English physician (1796) experimentally verified this belief. A boy was inoculated with the contents of a cowpox pustule from the hand of a milk-maid. Later the same boy was inoculated with the contents of a pustule from a case of human smallpox. This inoculation was followed by no inconvenience to the boy. Jenner's¹ own description of this event is given below.

"The more accurately to observe the progress of the infection, I selected a healthy boy (James Phipps) about eight years of age for the purpose of inoculation for the cowpox. The matter was taken from a suppurated sore on the hand of a dairy maid (Sarah Nelmes) who was infected by her master's cows, and it was inserted, May 14,

¹ *Lancet*, January, 1923, p. 137.

1796, into the arm of the boy, by means of two superficial incisions, each about $\frac{3}{4}$ of an inch long. On the seventh day he complained of uneasiness in axilla and on the ninth, he became a little chilly, lost his appetite and had a slight headache. During the whole of this day he was perceptibly indisposed, and had a rather restless night; but, on the day, following, he was perfectly well. The appearance, and progress of the incisions to a state of maturation, were pretty much the same as when produced in a similar manner by variolous matter. The only difference which I perceived, was, that the edges assumed rather a darker hue, and that the efflorescence spreading around the incisions, took on rather more of an erysipelatous look, than we commonly perceive when variolous matter has been made use of for the same purpose.

"On July 1 following, this boy was inoculated with matter immediately taken from a smallpox pustule. Several punctures and slight incisions were made in both his arms, and the matter was well rubbed into them but no disease followed. The same appearances only were observable on the arms, as when a patient has had variolous matter applied, after having either the cowpox, or the smallpox."

Active immunization against rabies, although the virus itself is unknown, is likely another instance of vaccination by an attenuated organism. In this case attenuation is apparently accomplished by desiccation together with a temperature below the optimum. The spinal cord of a rabbit, containing the virus, fixed at a maximum of pathogenicity, is dried over KOH, in a dark place at a temperature of 22° to 23° C. At the end of thirteen days or more pathogenicity has been practically lost. Such an innocuous cord is used to initiate the process of active immunization.

Often pathogenicity diminishes with increasing age of a culture. This method exposes the organisms to the cumulative action of their own metabolites, which always inhibit complete, normal functioning at least. The common illustration of this mode of attenuation is interesting because it was the first instance in which a cultivable and isolated bacterium was used for active immunization. Pasteur in his studies on chicken cholera, inoculated some hens with an old culture, put away and forgotten a few weeks before. The birds became ill but recovered. They were then inoculated with a fresh culture, of proved pathogenicity, but the phenomenon of resistance recurred. By putting between the cultures variable intervals of days, of one, two or three months, variations of mortality were obtained, 8 hens dying out of 10, then 5, then only 1

out of 10, and at last, when, as in the first case, the culture had had time to get stale, no hens died at all, although the microbe could still be cultivated. While hens which had never had chicken cholera perished when exposed to the deadly virus, those which had undergone attenuated inoculations, and which afterward received more than their share of the deadly virus were affected with the disease in a benign form, a passing indisposition; sometimes even, they remained perfectly well; they had acquired immunity.¹

Prolonged artificial cultivation, combined with the action of certain chemicals in the media, has been used for attenuation. Calmette and Guérin² secured an attenuated strain of bovine tubercle bacilli, after twelve years' cultivation on glycerinated media containing bile. This strain was avirulent for cattle, the ape, and the guinea-pig. These animals tolerated intravenous injection of even considerable numbers of the microorganism; and in no case were tuberculous lesions produced. Such animals were thereby rendered actively immune for a period of eighteen months against experimental inoculation and even against contamination of association with infected animals in infected stables.

Attenuation has been accomplished by cultivating the organisms at other than the optimum temperature range. Anthrax bacilli incubated at a temperature of 42° C. or 43° C. do not form spores. For instance, a strain which killed 10 sheep out of 10, when cultivated at that temperature for eight days killed only 4 or 5; "After ten or twelve days' cultivation it does not kill any; it merely communicates to animals a benignant malady which preserves them from the deadly form."³

Addition of chemicals to the medium in some instances, induced attenuation. Chamberland and Roux⁴ attenuated anthrax bacilli by growing them in the presence of phenol (1 to 600), K₂Cr₂O₇ (1 to 500), and H₂SO₄ (1 to 200). Under such treatment the bacilli lost the ability to form spores and became avirulent for sheep.⁵

In brief organisms may be attenuated by (1) animal passage; (2) by desiccation; (3) by prolonged artificial cultivation; (4) by growing in presence of its own metabolites; (5) by cultivation at temperature outside the optimum range; (6) by addition of anti-septics to the medium, as illustrated above and in addition (7) by

¹ Vallery-Radot: *Life of Pasteur*, 1915, p. 299.

² *Ann. d. l'Inst. Pasteur*, 1920, **34**, 553.

³ Vallery-Radot: *Life of Pasteur*, 1915, p. 311.

⁴ *Compt. rend. d. l'Acad. d. sci.*, 1882, vol. **96**.

⁵ Zinsser: *Infection and Resistance*, 1918, p. 66.

cultivation under increased atmospheric pressure.¹ In practice often two or more of the above factors are used at the same time.

(b) Active immunization by injection of living fully virulent organisms in non-lethal doses, or into a resistant locus.

This method is not at present widely used, though there is much in its favor. Its application to man is hindered by serious dangers. Ferran² has employed it against cholera in man and other animals. Fully virulent rabies virus has been used, starting with a dilution of 1 to 10,000 and rapidly working up to a dilution of 1 to 10.³ Animals have been actively immunized against anthrax⁴ by injecting a single thread (3 to 6 bacilli) of a twelve-hour agar culture as the first dose and then gradually increasing the amount.⁵

(c) Active immunization by the injection of toxins. Horses are actively immunized by this method in the preparation of antidiphtheritic and antitetanic sera. A bacteria-free filtrate, containing the soluble toxin, is injected into the animal. The toxin stimulates the tissues to produce actively the corresponding antitoxin.

A modification of this method has been in recent years introduced for the prophylactic active immunization of human beings against diphtheria. A mixture of toxin and antitoxin, slightly toxic for the guinea-pig, is used; 1 cc of this is injected subcutaneously into the susceptible (Schick positive) individual, once a week until three doses have been given. Sometimes a fourth or a fifth injection is necessary. It is presumed that the toxin-antitoxin complex dissociates in the body, thereby liberating free toxin which acts as the stimulus necessary for the production of specific antibodies. The associated antitoxin minimizes the harmful effects of the toxin. This method is very safe and very efficient. The reaction to the injections is far less than that seen in smallpox or typhoid vaccination. The general application of this method will make diphtheria as rare as smallpox or typhoid fever now are where available precautions have been taken.

(d) Active immunization by injection of extracts of bacterial cells.

The best known attempts which fall within this category are the studies of Robert Koch and others which led to the production of the "tuberculins:" "Old Tuberculin," "New Tuberculin (TR and TO)," etc.

¹ Chauveau: *Compt. rend. d. l'Acad. d. sci.*, 1884, vol. 98.

² *Compt. rend. d. l'Acad. d. sci.*, 1885.

³ Hoegyes: *Lyssa*, Nothnagel's *Handbuch*, Wien, 1897.

⁴ Webb, Williams and Barber: *Jour. Med. Res.*, 1909, vol. 15.

⁵ Zinsser: *Infection and Resistance*, 1916, p. 66.

(c) Active immunization by injection of killed organisms. Vaccins of this nature are also known as bacterio-vaccins and bacterins. This type is so generally used today that the word "vaccin" unless otherwise qualified is often assumed to refer to it alone. There are various kinds of the vaccins or bacterins. An autogenous vaccin is one made from an organism isolated from the very case to be treated. It is the consensus of clinical opinion that autogenous are preferable to stock vaccins. A stock vaccin is one made and kept in "stock" to be used prophylactically against or therapeutically for treatment of a condition *similar* to that from which the organism used in its preparation had been isolated. The organism used was isolated from some case other than the one in which the vaccin is employed. Obviously all vaccins used prophylactically must be stock vaccins. A mixed vaccin is either an autogenous or a stock vaccin, composed of more than one bacterial species. An univalent vaccin is composed of only one strain of a bacterial species, while a *polyvalent* vaccin is composed of a number of strains of a bacterial species. *Sensitized* vaccins are prepared by exposing suspensions of bacteria to moderate amounts of a strong immune serum, inactivated at 56° C. Before injection the bacteria must be washed free from the serum. In terms of Ehrlich's side-chain hypothesis, this process secures the union of antigen (bacterial suspension) with specific amboceptor before injection into the patient. This presumably facilitates the active immunization because to complete the reaction the patient has only to supply from his own resources complement or alexin. Sensitized bacteria are far more readily ingested by phagocytes than are untreated bacteria.¹ *Lipo-vaccins* consist of killed, dried bacterial cells incorporated in an oily vehicle; *e. g.*, olive or almond oil. After injection the absorption of antigen is thereby retarded, thus minimizing any toxic effects of the bacterial protein and prolonging the period of active immunization.

Georges Dreyer² has introduced the term, "defatted" vaccins, to describe bacterins deprived of much of their lipoidal content. This is done with the idea of facilitating the disintegration of the bacterial cell. Presumably the production of antibodies would be accelerated and quantitatively increased. This method is particularly adapted to acid-fast and Gram-positive microorganisms. Practically the same end has been achieved by Arima, Aoyama and Ohnowa.³ These authors secured non-acid-fast tubercle bacilli by

¹ Zinsser: Infection and Resistance, 1918, p. 68.

² Brit. Jour. Exper. Path., 1923, 4, 146.

³ Deutsch. med. Wehnschr., 1924, 50, 666.

cultivation on saponin-containing media. Such strains possessed low virulence and protected rabbits and guinea-pigs against serious results of subsequent injections of more virulent strains. Price¹ has observed in his own experience in the treatment of oral infections and their sequelæ many instances where "defatted" vaccins apparently possessed a distinct advantage over those made in the usual way.

The question of the rationale of the use of vaccins in the treatment of localized infections constantly comes up. It is often asked, if the microorganisms are already in the tissues of the patient, what can be gained by artificially introducing a few more? The answer is that the absorption of antigens from the lesion, *e. g.*, a boil, is low or *nil*. In favor of this interpretation are the findings of Opie² that the absorption of antigens from an inflamed region is much less than from a non-inflamed region. Sometimes this absorption is suppressed. The antigen is fixed at the site of the local lesion. This fact has been experimentally demonstrated. In such a case, of course, antibody formation would be low, and the separate, artificial introduction of the antigen into the system, as is done in vaccinotherapy, is rational because in this way the antibody content of the body fluids is raised. This result will be conducive to an amelioration of the local lesion.

PREPARATION OF BACTERIAL VACCIN.

The preparation of a bacterial vaccin or bacterin may be considered under the following headings:

1. Isolate and identify organism.
2. Secure an abundant growth of it (preferably not over twenty-four hours old).
3. Suspend the growth in sterile physiological (0.85 per cent) sodium chlorid solution.
4. Kill organism.
5. Estimate number of bacteria per unit volume (the cubic centimeter, cc). This step may and sometimes must precede No. 4.
6. Dilute to desired dosage. The diluent may be 0.2 per cent tricoresol in physiological salt solution.
7. Test for sterility of vaccin; inoculation of the product of the sixth step onto suitable culture medium or into a susceptible animal.

¹ Dental Infections, 1923, **1**, 537.

² Jour. Exper. Med., 1924, **39**, 659, and Jour. Immunol., July, 1924.

Isolation and Identification of the Organism.—The theoretical basis on which bacterin therapy rests, requires the employment of the species or strain which is responsible for the condition to be treated or against which protection is desired. This is why the preference is almost always given to autogenous vaccins. In the first place the primary cultures must have been taken correctly and the cultural conditions must be suitable for the growth of the causative agent or agents. Under these conditions a number of different kinds of bacteria may be isolated. This is particularly likely to happen when dealing with lesions of the skin, respiratory or alimentary tracts; for example this usually occurs in cultures from pyorrhea alveolaris. The problem then is to determine which of these bacteria is (or are) of etiological importance. For this purpose, in the case of pyorrhea alveolaris, Goadby and the other early workers relied on the opsonic index. Bulleid¹ has found in his experience that agglutination tests give the most satisfactory results. Cutaneous tests to determine the hypersensitivity of the patient to one or another of the bacteria isolated have been employed by some, but have not come into general use. Gordon² incubates the isolated organisms with successive dilutions of normal human serum and then plants out each dilution on plates on appropriate media. Generally speaking, the strains that resist human serum in high concentration are pathogenic. He is certain that vaccins prepared from these serum-resisting strains are of much greater clinical value. This method is similar to one proposed by Solis-Cohen and Heist³ in which, however, the patient's whole blood is used. They consider that when an organism grows in the fresh, whole, coagulable blood of the infected individual, there is strong presumption that it is the etiological organism or a complicating organism, or is likely to become the latter. On the other hand, when an organism present in an infected discharge, secretion, or excretion, or on an infected area, disappears when planted in the whole blood of the infected individual, it may be fairly presumed to have no part in the infection. Its inclusion therefore in an autogenous vaccin may be regarded as unnecessary and possibly harmful. The technic is very simple.⁴ A small quantity of the infected material, collected with proper precautions against contamination, is put in the bottom of a rather wide test-tube (20 mm. or more inside diameter). Five cc or more of blood is aseptically collected from the

¹ Dental Surgeon, 1924, p. 314.

² Lancet, 1924, **206**, 1130.

³ Pennsylvania Med. Jour., 1921, **25**, 27.

⁴ Clark, J. H.: Jour. Lab. and Clin. Med., 1924, **10**, 243.

vein of the patient in a sterile syringe and at once transferred to the test-tube. The mixture is incubated for eighteen to twenty-four hours and a vaccin is prepared from the organism or organisms that survive. The relative quantities of inoculum and blood are apparently unimportant as long as there is a great excess of blood.

Killing the Organism.—This is a very important step. It is essential to select a method which preserves as much of the immunizing properties of the virulent, living organisms as possible. In the preparation of bacterins, reliance is usually placed on heat. The bacterial suspension in a sealed glass ampoule is entirely immersed in a water-bath. The temperature and the time of exposure vary for different species. For example, Russell in making typhoid vaccins for the United States Army subjected the suspension of *B. typhosus* to 53° C. for one hour.¹ Haffkine,² in prophylactic immunization against bubonic plague used broth cultures killed at 65° C. Cholera cultures, killed at 58° C. for one hour have been employed by Kolle³ and by Pfeiffer and Marx.⁴

A temperature of 65° to 70° C. for thirty minutes suffices to kill most asporogenic bacteria without too seriously affecting their antigenic (immunizing) properties. Many workers do not raise the temperature above 60° C. In this case, careful controls for sterility must be run. The best results have been obtained when the temperature was not higher than 33° to 55° C.⁵

Antiseptics have also been used, alone or in conjunction with heat, to kill bacteria for vaccins; *e. g.*, phenol, 0.5 per cent; toluol (removed before injection by filtration or evaporation); chloroform; formaldehyd, 1 per cent, and alcohol.

Increased atmospheric pressure has also been suggested for the killing of bacteria in the preparation of vaccination.⁶

Zivy⁷ puts the bacterial suspension contained in an aluminum tube, in a freezing apparatus with a constant temperature of minus 18° C. This temperature is obtained in the interior of the aluminum tube in about one hour and a half. The tubes are left in the freezing apparatus for five hours, taken out and left another five hours at room temperature (about 16° C.). Thus freezing and thawing has taken place. According to the species of bacteria this opera-

¹ Zinsser: *Infection and Resistance*, 1918, p. 485.

² Bull. d. l'Inst. Pasteur, 1906, 4, 825.

³ Deutsch. med. Wchnschr., 1897, p. 4.

⁴ Ibid., 1898.

⁵ Zinsser: *Loc. cit.*, 1918, p. 68.

⁶ Larson, Hartzel and Diehl: *Jour. Infect. Dis.*, 1918; reprinted in *Jour. Nat. Dent. Assn.*, April, 1918, p. 396.

⁷ *Lancet*, 1924, 206, 221.

tion must be repeated twice for pneumococci and streptococci, four times for *B. coli*, and six times for staphylococci and enterococci. On completion of the requisite number of periods in the freezing apparatus, sterilization is complete. The original suspension is then diluted with physiological salt solution in order to obtain the required dosage.

Estimation of Number of Bacteria.—There are several ways in which the number of bacteria present in a unit volume of the suspension may be estimated.

(a) When this method is employed, it must be used before the organisms are killed. A given volume of the suspension is diluted with a given volume of sterile bouillon or sterile 0.85 per cent NaCl solution. A given volume of this mixture is plated on agar. The plates are incubated. The colonies which develop are counted. On the assumption that each colony represents a single bacterial cell in the original suspension, the number of organisms present in a given volume of that suspension may be calculated with proper corrections for the dilutions. The weakness of this method is that the assumption that each colony developing on the plates represents a single bacterial cell in the original suspension, is not justified by fact. Often such colonies represent a small clump of organisms. The estimates by this method tend consequently to be low.

(b) Comparing the opacity of the bacterial suspension with standard suspensions, whose equivalents in numbers of bacterial cells per unit volume have already been determined. The standard suspensions may contain some inorganic precipitate, *e. g.*, BaSO₄ or actual bacterial cells preserved by formalin or some other fixative. Dreyer¹ uses a standardized suspension of chicken erythrocytes. Determining the concentration of suspensions by comparison of opacities is known as nephelometry (*nephele*, Gk. = mist). Instruments to facilitate this comparison are known as nephelometers. Apparatus and manipulation are detailed by Hans Heckscher.²

(c) A given volume of the suspension is added to a graduated tube (Fig. 66), which is then centrifugalized at a given speed for a given time. The suspended particles (bacteria) are thrown to the bottom of the tube. The tube has been standardized so that each mark represents a certain number of bacteria. The mark up to which the precipitated particles reach, indicates the number of bacteria in a given volume of the bacterial suspension.³

¹ Lancet, January, 1921, p. 219.

² Compt. rend. Soc. de biol., Paris, 1921, 85, 378.

³ Hopkins: Jour. Am. Med. Assn., 1913, 60, 1615.

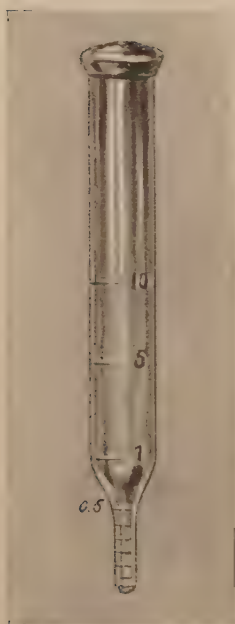


FIG. 66. — Hopkins' tube for standardizing a bacterial vaccine. (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

(d) The most widely used method of estimating the concentration of bacterial suspensions is to determine the bacterial-erythrocyte ratio (Wright). It requires a small drop of blood from an individual whose erythrocyte count is approximately known. For illustration, the normal adult male count of 5,000,000 per cm. may be taken. The unit volume of bacterial suspension is usually 1 cc. Since there are 1000 cubic millimeters in 1 cc., there will be 5,000,000,000 erythrocytes per cubic centimeter of blood.

A small capillary glass pipette is taken (Fig. 67). About 2 cm. from its tip a mark is made on it with a paraffin-pencil. Over the wider opening of the pipette is placed a small rubber bulb. Into this pipette, the length of whose capillary part is about 15 cm., bacterial suspension is drawn to the blue mark. Then a small drop of air is taken in. Then

from a prick on the finger enough blood is sucked in to come to the blue mark. Then a second small drop of air and then small quantity of some isotonic liquid (citrate or NaCl solution). There is no need to measure exactly the quantity of this liquid. It is to act as a diluent to give a workable volume of liquid and to prevent coagulation of the blood.

At this stage the capillary pipette contains (1) 1 volume of bacterial suspension, and separated from this by little cushion of air (ca. 1 cm. long), (2) 1 volume of blood of known erythrocyte count, and separated from this by a second little cushion of air a variable quantity of diluent. The pipette is emptied into a small watch-glass and its contents thoroughly mixed by repeatedly sucking up



FIG. 67.—A capillary pipet for counting a bacterial vaccine. This illustration shows the pipet loaded with three equal volumes of sodium citrate solution, blood and bacterial emulsion in proper order. (Kolmer: Infection, Immunity and Biologic Therapy, W. B. Saunders Company, Publishers.)

into the pipette and blowing out again. A small drop of this mixture is spread over a clean slide, as in making blood smears for differential counts. The film is dried and stained by some blood stain, *e. g.*, Wright's, Jenner's, or Giemsa's. The stained slide is examined with the oil-immersion lens (Fig. 68). The number of bacteria and the number of erythrocytes seen in a certain number of microscopic fields, are counted. To facilitate the counting, a small circle is drawn with a paraffin pencil on the lower, larger lens of the ocular; and the area within this circle taken as the microscopic field.

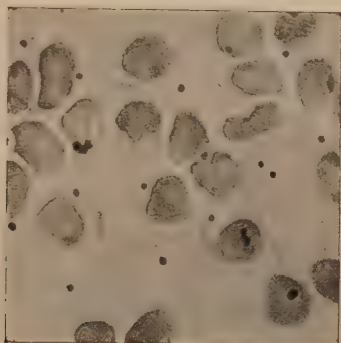


FIG. 68.—Counting a bacterial vaccine after the method of Wright. (Kolmer: *Infection, Immunity and Biologic Therapy*. W. B. Saunders, Company, Publishers.)

The following hypothetical calculations serve to illustrate the working of this method. If 800 bacteria and 500 erythrocytes were counted, then since equal volumes of blood and of bacterial suspension were used, the bacterial erythrocyte ratio would be 8 to 5. Since in 1 cc of blood there are 5,000,000,000 erythrocytes, there are accordingly 8,000,000,000 bacterial cells in 1 cm. of the original suspension. This calculation is nothing but the elementary "rule of three" and may be expressed as follows:

$$E : B :: E^1 : X$$

E = number of erythrocytes per microscopic field.

B = number of bacteria per microscopic field.

E^1 = number of erythrocytes per cubic centimeter of blood.

X = number of bacteria per cubic centimeter of suspension.

(*e*) Leishman¹ in his studies on typhoid vaccinations standardized his suspensions by weighing the dried residue of a given volume of the suspension. Wilson and Dickson² give the details of a rapid gravimetric method of standardization.

¹ Jour. Hyg., 1908, p. 384.

² Ibid., 1912, 12, 49.

(f) The most accurate method of estimating the concentration of bacterial suspensions is to make a direct count of the number of bacteria in a given volume. For this purpose is used an ordinary hemocytometer, such as is used in making erythrocyte or leukocyte counts, or a special counting chamber, devised for bacterial counts (Helber chamber). (Fig. 69.) The principle of these special chambers is in no way different from that of the ordinary hemocytometer. Mallory and Wright used a blood-platelet counting chamber.

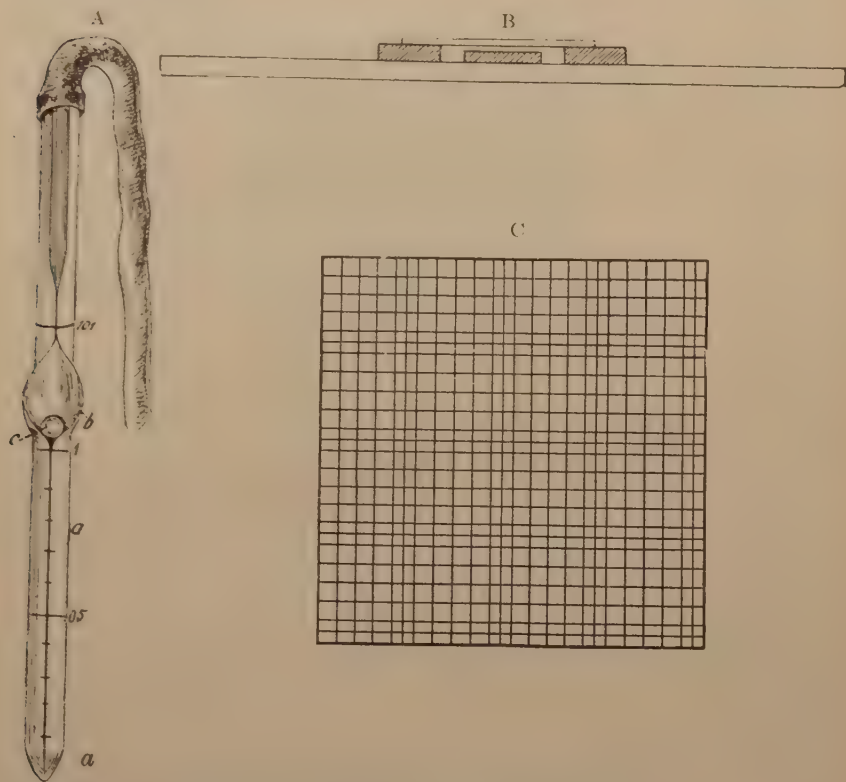


FIG. 69.—Thoma-Zeiss blood-counting apparatus (Limbeck). A, pipette for diluting blood in making count of red corpuscles; B, cross-section of the chamber in which the blood corpuscles are counted; C, diagram of the middle millimeter square.

Dilution to Desired Dosage.—Formulae have been devised to facilitate the dilution of the killed suspension to the desired dosage:

$$\frac{S}{V} - 1 = Q$$

S = number of bacteria in the unit volume of the suspension.

V = number of bacteria desired in the unit volume of the vaccin.

Q = number of volumes of diluent (e. g., 0.2 per cent tricresol in sterile physiologic sodium chlorid solution) required to produce desired result (V).

To illustrate: assuming that $S = 8,000,000,000$, and $V = 500,000,000$.

$$\frac{8,000,000,000}{500,000,000} - 1 = Q = 15$$

This means that 15 cc of diluent must be added to each 1 cc of the suspension in order that each cubic centimeter of the resulting mixture will contain 500,000,000 bacteria.

II. Another formula¹ is:

$$\frac{N \times V}{S} = s$$

N = number of cubic centimeters of diluted suspension (vaccin) required.

V and S = same values as in formula I.

s = number of cubic centimeters of the undiluted suspension which must be added to $(N-s)$ cubic centimeters of diluent to give a mixture, each of which will contain the number of bacteria indicated by V .

To illustrate: assuming that $N = 30$: $V = 500,000,000$: $S = 10,000,000,000$.

$$\frac{30 \times 500,000,000}{10,000,000,000} = s = 1.5$$

$$N-s = 28.5.$$

This means that if 1.5 cc of the undiluted suspension be added to 28.5 cc of diluent, we shall have 30 cc of vaccin each cc of which will contain 500,000,000 bacteria.

Vaccin Dosage.—Kolmer² gives the following values for V of the above formulæ.

Staphylococci	1,000,000,000
Streptococci	500,000,000
Pneumococci	500,000,000
Gonococci	500,000,000
M. catarrhalis	500,000,000
B. typhosus	1,000,000,000
B. paratyphosus	1,000,000,000
B. coli	1,000,000,000
Pseudomonas pyocyanea	1,000,000,000
B. influenzae	1,000,000,000

These values hold for vaccins composed of a single species of microörganism. Where mixed vaccins are used, it is customary to add equal numbers of each species so that the total will be approximately 1,000,000,000 bacteria per cubic centimeter.

For the use of vaccins in the treatment of pyorrhea alveolaris, Nathaniel Gildersleeve³ gave the following dosages: *Staphylococcus*

¹ Drennan: Jour. Am. Med. Assn., 1923, **81**, 929.

² Infection, Immunity, etc., Philadelphia, 3d ed., 1924, p. 198.

³ Kirk's American Textbook of Operative Dentistry, 4th ed., 1911, p. 509.

aureus or *albus*, 50,000,000 to 600,000,000; *Streptococcus pyogenes*, 10,000,000 to 100,000,000; pneumococcus, 10,000,000 to 100,000,000; *M. catarrhalis*, 25,000,000 to 100,000,000; and *B. influenzae*, 25,000,000 to 100,000,000.

Bacterins are usually administered by subcutaneous injection. Under the dorsal margin of the deltoid muscle is a favorite site. The skin should be cleansed, dried, painted with tincture of iodine and then washed off with about 95 per cent ethyl alcohol. The frequency of the injections should be every three or four days unless contraindicated by unfavorable reactions. In this latter case the interval may be lengthened or the volume of vaccin injected may be reduced. The initial dosage is usually about 0.2 cc. This is gradually increased on subsequent injections until 1 cc is given. The concentration of the vaccin should be such that larger volumes are not necessary. There is no general rule as to the number of injections necessary when vaccins are used therapeutically. If obvious clinical improvement sets in early, the injections are continued for a short time after the disappearance of the symptoms. If clinical improvement be apparently absent or uncertain, it is difficult to decide when to give up this form of treatment. Usually the tendency seems to be to give it up too soon.

Besredka whose studies have impressed him with the importance of the local response of tissues to bacterial damage, advocates the introduction of the bacterin at the affected or susceptible site.

BACTERINS IN ORAL INFECTIONS.

It is well to introduce this phase of the discussion by pointing out that bacterins may be employed in oral infection with two purposes in mind. These may of course be often interdependent but it is simpler to regard them arbitrarily as distinct. In the first place, the bacterin is administered with the hope of ameliorating the *local oral* lesion. In this case it is ethical and permissible for the properly trained dentist to administer the vaccin. On the other hand, the sole or chief aim of the vaccinothrapy may be to aid in the cure of some lesion anatomically remote from, but regarded as secondary to, the oral infection. In this case, although it lies well within the province of the dentist to make the cultures from the oral lesion from which the vaccin is to be prepared, the vaccin itself should not be administered by the dentist but by the physician in charge. This attitude is a simple inference from the ethical and legal considerations of the case.

It is of course apparent that a vaccin may be used at once for both the above purposes. There can be no objection to the dentist hoping that by clearing up an oral lesion some improvement in the patient's general condition will occur. In considering the value of vaccinotherapy it is necessary to keep in mind various possibilities; the vaccin might induce improvement of the oral lesion, or it might induce improvement of a remote lesion or of the general condition of the patient with or without altering the state of the oral lesion.

Staphylococcal bacterins have been used with apparent advantage in cases of chronic osteomyelitis of the mandible. In these cases, the establishment of drainage and the removal of sequestra must not be omitted.

Actinomycosis has been treated by corresponding vaccins. Wynn¹ reported a case of lung and liver infection, cured by vaccin therapy. Similar cases have been reported by Collie,² by Malcolm³ and by Dean.⁴ Cope⁵ has found the use of vaccins a valuable adjunct in treatment. Not only the primary agent but such bacteria as are secondary invaders should be included in the vaccin. Initial doses should be small, *e. g.*, about 500,000 actinomycetic fragments and may gradually be raised to more than 50 million. The common interval between injections is from three to seven days. Henrici and Gardner⁶ used an autogenous actinomycetic vaccin but it is impossible to evaluate its influence on the course of the disease. These authors describe carefully their technic of vaccin preparation; a troublesome problem in the case of these microorganisms. The organism was grown in flasks on broth containing 3 per cent each of dextrose and glycerol, until the pellicle broke, after about ten days. It was then heated to 60° C. for one hour, and placed in Dr. Larson's apparatus under the pressure of a fresh tank of carbon dioxid over night. The resulting suspension was uniform and viable. It was again heated to 65° C. for an hour and 0.25 per cent of tricresol was added, when it was found to be sterile. Colebrook⁷ reported on 23 cases of actinomycosis treated by vaccins, always in association with simple surgical measures for adequate drainage. He is convinced that the use of vaccins facilitates recovery under these conditions. Generally the treatment was commenced with 2,000,000 or 2,500,000 mycelial fragments at intervals of five days.

¹ Brit. Med. Jour., March 8, 1908.

³ Ibid., 1916, ii, 488.

⁵ Brit. Dent. Jour., 1920, 41, 649.

⁷ Lancet, April 30, 1921, p. 893.

² Ibid., 1913, i, 991.

⁴ Ibid., 1917, i, 82.

⁶ Jour. Inf. Dis., March, 1921, 28, 232.

The dose was gradually increased. The best results occurred with 4,000,000 to 10,000,000 fragments. Later Colebrook standardized his vaccins in terms of the weight of dried culture in a given volume of the suspension. The advisable procedure would seem to be to commence treatment at once upon diagnosis with a polyvalent stock vaccin and then, as soon as prepared, to substitute therefore an autogenous vaccin.

Tunnick¹ found that the injection of killed cultures of fusiform bacilli and spirochetes in a case of noma caused an increase in the amount of specific opsonin and appeared to produce some beneficial result.

Rogers² employed autogenous streptococcal vaccins in 17 unselected and as consecutive as possible cases of sprue. The bacteria were isolated from the oral cavity. The initial dose was about 50,000,000. This was increased at five-day intervals to 200,000,000. Late in the treatment the interval between injections was ten days. In most instances the treatment continued for three to six months. The author found that the results in cures or in marked improvement were strikingly superior to results obtained by other methods.

In 3 chronic cases of alveolar abscess, Gilmer and Moody³ employed autogenous vaccins with strikingly beneficial results. The vaccins were prepared from aerobic and anaerobic cultivations and were administered in graded doses at five-day intervals. The cases were the chronic suppurative conditions which follow that class of acute infections of the mandible characterized by much brawny swelling, persisting over a week or more with little or no indication of "pointing." The pus discharge continued with no seeming diminution for several weeks after the acute symptoms had subsided. In each instance the pus flow stopped promptly on the use of the vaccin. Daland⁴ employed an autogenous streptococcal vaccin in a case of ulcerative endocarditis which he regarded as secondary to dental sepsis. The streptococcus was isolated, *via* the root canal, from a periapical infection. Other periapical, potential foci existed. Large doses did harm. The vaccin treatment was continued for two months and exerted no recognizable beneficial effects upon the fever or other symptoms.

Head and Roos⁵ give a partial description of an anaerobic "cocco-

¹ Jour. Infect. Dis., 1911, **8**, 316.

² Indian Med. Gaz., 1918, **53**, 121.

³ Jour. Am. Med. Assn., 1914, **63**, 2023.

⁴ Med. Clin. North America, September, 1917.

⁵ Jour. Dent. Res., 1919, **1**, 13.

bacillus" isolated quite constantly from periapical infections. "Bacterial vaccins containing this organism and streptococci seem to have given far more permanent results, although the dosage had to be greatly reduced."

Cahn¹ notes that vaccins are indicated in osteitis of the tooth-socket ("dry socket"), in the slow healing of operative wounds and in the pericoronal infections about mandibular third molars.

Vaccins in Pyorrhea Alveolaris.—The most extensive application of bacterins to the treatment of oral infections has been made in the case of *pyorrhea alveolaris*. Before discussing this topic it is desirable to make a confession of faith, as it were, regarding the pathogenesis of the group of affections generically included in this term. It seems well established by the researches and experiences of Talbot, of Hopewell-Smith² of Roy³ and of Gottlieb and Fleischmann⁴ that the underlying, predisposing factor in these conditions is of paramount importance and that this factor is the precocious, senile atrophy of the alveolar margin. This atrophy may have been determined by various "causes," such as the anemias or occlusal trauma. The infection which may then become implanted upon the gingival and periodontal tissues is to be regarded as a secondary phenomenon. This is no less true if we accept Box's⁵ view that the initial, significant lesion in these periodontal troubles is a "rarefying pericementitis fibrosa." To say that the consequent infection is secondary, is however, not the same as saying it is unimportant and negligible. In fact wherever "pyorrhea" in its etymological sense, as a "flow of pus," exists we have infection which aggravates the primary local condition, complicates the treatment and is solely responsible for the remote or systemic symptoms which give general significance to this group of oral lesions. In brief, whatever be one's views on the etiology of "pyorrhea alveolaris," the existence of an infectious factor must be granted and consequently it is not irrational to include means of combating this factor in one's campaign against the clinical complex, known as "pyorrhea alveolaris." It is therefore not surprising that early in the history of bacterin therapy attempts were made to treat "pyorrhea alveolaris" (in which pyogenic bacteria are so obviously present) by means of vaccins. It must not be forgotten that these should constitute only a part of the treatment, directed against a

¹ Modern Practice of Tooth Extraction, New York, 1924, p. 109.

² Dent. Cosmos, 1911, 53, 397, 981.

³ Ibid., August and September, 1918.

⁴ Ibid., 1921, 63, 215, 375.

⁵ Canadian Dent. Assn., Bull. No. 7, 1924.

secondary symptom and not against the primary factors. Just as vaccins have been satisfactorily employed, supplementary to drainage, curettage and removal of sequestra in cases of pyogenic osteomyelitis of the mandible or of actinomycosis, so it is illogical to place sole reliance on vaccins in cases of "pyorrhea alveolaris." If used at all with the intention of ameliorating the oral conditions, they must form only one item of the treatment. Calculus must be removed, occlusal trauma relieved, temporary or more or less relatively permanent immobilization of the involved teeth must be achieved, attention to the condition of the pulp and periapical tissues must be given, correction or control of systemic disturbances *e. g.*, diabetes mellitus or the anemias, must be attempted, surgical obliteration of the pyorrhetic pocket performed where indicated, and the coöperation of the patient in the way of conscientious, intelligent, simple mouth cleanliness must be secured.

On this account it is very difficult to evaluate the part played by vaccins in any local improvement observed. The personal equation, the bias of the operator, which of necessity enters largely into the conclusions drawn, is even with the best conscious intentions notoriously misleading. In view of the conditions indicated above, under which vaccin therapy must be carried out in cases of "pyorrhea alveolaris," it is small wonder that there exists diversity of opinion as to its worth. At the present time the opinion of most critical and experienced clinicians seems to be that the use of bacterial vaccins is superfluous so far as any favorable influence they may exert upon the course of the local lesion is concerned. With regard to the prophylactic or therapeutic worth of these agents toward remote sequelæ of the oral infection, clinical opinion of the physicians is less definitely crystallized. The question is still generally considered as *sub judice*, and vaccin therapy is often advocated in the spirit that it will do no harm and may do some good.

It must be remembered, however (and this applies to the possible effects, both local and systemic), that in such matters a preponderance of negative results, even if admitted for the sake of argument never can *ipso facto* or by inference invalidate such positive results as may from time to time be observed.

As will shortly appear a rather large body of evidence, favorable to the use of bacterins in oral pyorrhetic infections, exists. At least some of this evidence has been reported by observers, admittedly experienced and of critical spirit. Under the circumstances, the author is of the opinion that so little systematic thought and

labor has as yet been given to the subject that we would be unjustified in discarding at the present time such a theoretically useful weapon as vaccin therapy.

Goadby probably has had a longer experience than any one else in the use of bacterins for oral infections. In 1905¹ he reported a study of about 20 cases of pyorrhea alveolaris and found staphylococci frequently, associated with a low opsonic index. The administration of autogenous vaccins was followed by a rise in the opsonic index and an amelioration of the general and local symptoms. In 1910, Goadby² summarized his experiences with 70 cases of early pyorrhea alveolaris. The methods of treatment were threefold: (1) Autogenous vaccins, the antigen of which had been associated with a significant opsonic index; (2) local treatment, including lavage and syringing, surgical interference where indicated and extirpation of any pockets by actual cautery; (3) medicinal treatment where indicated, *e. g.*, arsenic or in anemia, iron and arsenic. It is suggested that the local treatment had best be given only after a preliminary immunization. The reason for this would be to minimize secondary localizations, arising from such bacterial emboli as might occur in the manipulation of the infected soft parts. Forty-five of the 70 cases treated as above were cured. In 13 relief was obtained; 11 cases disappeared during treatment; 1 case died. Goadby asserts that cure is possible in over 60 per cent of early cases of pyorrhea alveolaris when treated as above, while without vaccin treatment the disease will advance progressively in the majority of cases. A year later³ he reported on 45 cases of rheumatoid arthritis and other forms of rheumatism, presumably of oral origin. Autogenous vaccins of *Streptobacillus malæ* and local oral attention were used. Twenty-three of these cases were cured on the criterion that arthritic symptoms disappeared and the erstwhile patient could resume his normal vocation. In succeeding years Goadby's attitude toward vaccin in oral infections has not essentially changed. They are recommended in a large number of instances in his *Diseases of the Gums and Oral Mucous Membrane*.⁴ Gratifying results have been obtained in cases of neuritis with an autogenous streptococcal vaccin from the oral suppuration. In cases of chronic gingivitis and stomatitis with secondary pneumococcal or streptococcal infection, vaccin should be used. The same holds good for chronic hypertrophic gingivitis with

¹ Brit. Med. Jour., 1905, **ii**, 562.

² Proc. Roy. Soc. Med., Odont. Sect., 1910, **3**, 55.

³ Lancet, 1911, **i**, 639.

⁴ Oxford Med. Pub., 1923.

involvement of the whole alveolar margin. The indication, to be noted below, for vaccin administration in cases of "chronic alveolar osteitis" (presumably "pyorrhea") is very pertinent. As noted above in the summary of his 1910 report in which a preliminary immunization was advised before instituting instrumentation or manipulation of the soft parts, so in the 1923 publication (p. 116) we find the same recommendation applied in the case of such extractions as are necessary when we encounter the coëxistence of chronic disease which may fairly be attributed to the oral infection. Three to four injections at least of an autogenous vaccin, increasing in dose from 10,000,000 to 50,000,000, are to be given at five-day intervals.

Carmalt-Jones and Humphreys¹ employed autogenous streptococcal vaccins in the treatment of 4 cases of slight pyorrhea alveolaris. No local treatment was routinely given except ordinary cleansing. The authors believe that pyorrhea alveolaris can in some cases at any rate be much improved and even cured by the use of autogenous vaccins. Their frankness commends itself because in the first case the vaccin brought no improvement, although subsequent vigorous local treatment brought decided improvement. In the other 3 cases the vaccin seemed to be satisfactory. In the second case very marked improvement was noticeable within twenty-four hours. In the fourth case not only was the local condition improved but also the general (dyspepsia).

Friel,² from the results observed in 3 cases, was very favorably impressed with autogenous vaccins. Streptococci and staphylococci were employed. At least five injections were given, and the dosage ranged from 50,000,000 to 750,000,000. He felt sure that appreciable improvement in the local condition occurred even in the absence of all local treatment.

Beebe³ used vaccins in 17 cases of pyorrhea alveolaris, most of which had been referred to him as intractable to local treatment. Organisms which gave a low opsonic index were employed. The standard minimum dose was 250,000,000 staphylococci with 50,000,000 pneumococci, repeated every three to five days. Following the administration of the vaccin an immediate improvement in the patient's local and general condition was noticeable.

One of the classical reports on the use of vaccins in pyorrhea alveolaris is that made by Eyre and Payne.⁴ Thirty-three cases

¹ *Lancet*, 1907, ii, 1818.

² *Transvaal Med. Jour.*, 1908, 3, 286.

³ *Boston Med. and Surg. Jour.*, 1909, 161, 613.

⁴ *Proc. Roy. Soc. Med., Odont. Sect.*, 1909, 3, 29.

were bacteriologically studied. Of these 26 were systematically treated with autogenous vaccins. The responsible organisms were determined by the opsonic test, in which the patient's blood was used against the bacteria isolated. A significant index was considered to be 0.5 or lower, or 1.3 or higher. The cases were selected with reference to the following points: (a) Acuteness and intensity of the suppurative process; (b) extent of the disease and the number of teeth involved; (c) the *intractable* character of the disease and resistance to all ordinary methods of treatment. The organisms employed for the bacterins were *Staphylococcus aureus*, the *pneumococcus*, *Micrococcus catarrhalis*, *Streptococcus pyogenes longus* and the last two together. The results were very encouraging. Twenty are reported as cured so far as their local oral condition is concerned. "Cure" is constituted by the affected teeth being so firm that mastication is painless, and by no pus being expressible from around the necks of the teeth. Such cures were maintained for at least from six to fifteen months after discharge. Five cases are noted as "improved" in respect to their oral condition. The general condition of the patients whose pyorrhea cleared up under vaccin treatment also underwent decided improvement. In no less than 17 arthritic pains had been reported before the vaccin treatment was instituted; headaches, fatigue, lassitude, mental depression and "indigestion" were also noted. By the time the patient was examined when a "cure" was recorded, *i. e.*, six to fifteen months after cessation of treatment, most of these rather indefinite complaints had disappeared and the general condition was remarkably improved. In most instances the patient felt "quite well."

MacWatters¹ treated 48 cases of pyorrhea alveolaris with vaccin. Thirty of these cases completed the treatment; 21 of the 30 showed no return of the pus during at least two months after the cessation of treatment. The remaining 9 were greatly improved. The word "cure" in this report is apparently indicative of failure to find pus on several examinations. Autogenous vaccins were used when possible, otherwise polyvalent. The organisms used were streptococci, *Staphylococcus aureus*, *Micrococcus catarrhalis*, the pneumococcus and a leptothrix. The dosage was usually 5,000,000, repeated every eight days. The majority of cases need treatment for six months or more. Fortnightly injections of the vaccin are advisable for some months even after the disappearance of the pus. The vaccin treatment was supplemented by instrumentation, the

¹ Proc. Roy. Soc. Med., Gen. Repts., 1910, 3, 172.

practice of oral hygiene and the use of a hypertonic mouth wash. This last agency is a promising refinement to which attention will again be called. It is introduced with the intention of inducing an osmosis of opsonized lymph into the diseased gingival tissues and pockets. The wash advocated by this author consists of 4 per cent NaCl plus 0.5 per cent sodium citrate, flavored to taste. MacWatters is very sure that vaccins will clear up long-standing cases of pyorrhea after years of futile treatment by older methods. ". . . the results in about 75 per cent of my cases have been so successful, and the improvement in constitutional conditions so marked, that I feel confident in advising the adoption of the method (vaccin therapy) in all cases of pyorrhea alveolaris."

Murrell¹ employed an autogenous vaccin in a case of chronic septic arthritis. The patient has suffered for over twelve months with a pyorrhea alveolaris which had not been arrested by dental treatment. *Streptococcus brevis* and *M. catarrhalis* were isolated from the oral lesions and incorporated in equal parts into the vaccin. The initial dose was 50,000,000, rising to 200,000,000. After twelve injections the joint conditions were greatly ameliorated. No mention is made of the local oral condition.

Wallis² used autogenous vaccins in 2 cases of pyorrhea alveolaris which had proved unyielding to ordinary dental treatment. In the first case the organisms were a streptococcus and *M. catarrhalis*. The vaccin was supplemented only by massage and a mouth wash of KCl O₃. After twelve doses the pus flow had practically ceased. In the second case the pneumococcus was used. No local treatment accompanied the vaccin. After twelve injections no pus was demonstrable.

Williams³ studied the effect of vaccin treatment on two groups of patients with pyorrhea alveolaris. In the first group, in which autogenous vaccins were used, were 8 intractable cases of long standing. They had been carefully, though unavailingly, treated by dentists with the usual methods. The vaccin contained streptococci and staphylococci. The local benefit and the improvement in general health were very satisfactory. Thirteen dispensary patients comprised the second group, to which a stock vaccin was administered. No dental treatment was employed. In spite of this, Williams found the results on the whole encouraging.

Sibley⁴ reported 2 cases of pyorrhea alveolaris, associated with skin lesions. He employed an autogenous vaccin but it is impos-

¹ Practitioner, 1910, **85**, 637.

² Brit. Dent. Jour., 1911, **32**, 107.

³ Am. Jour. Med. Sci., 1911, **141**, 666.

⁴ Proc. Roy. Soc. Med., Odont. Sect., 1911, **4**, 71.

sible to form any clear idea as to his opinion concerning the relative value of this agency in the improvement.

Harris¹ employed in 1 case of pyorrhea an autogenous vaccin together with mild laxatives. The organisms were *Staphylococcus aureus* and *S. albus*. He was favorably impressed with what he regarded to be the effect of the vaccin.

Glynn² reported on 1 case, giving a history of bad conditions (pyorrhea alveolaris, presumably from the title) and general debility. An oral, autogenous streptococcus vaccin was given, 10,000,000 to 20,000,000 once a week for nine weeks. One tooth was extracted. There was no other treatment whatever. Most of the teeth became much tighter and improvement in the general condition was noted.

McGehee³ made a rather elaborate and systematic study of the use of stock vaccins in 30 cases of pyorrhea alveolaris. Each cubic centimeter contained: Streptococci, 50,000,000; *B. coli communis* and the pneumococcus, each 100,000,000; *Staphylococcus aureus*, *albus* and *citreus*, 500,000,000.

A group of 18 patients presenting pyorrhea of the first variety (Burchard's classification) were divided into three classes of 6 each, receiving the treatment as indicated below.

Class I.—Routine local and constitutional, but no vaccin.

Class II.—No local or constitutional treatment; vaccin (0.5 cc, four injections at weekly intervals) alone.

Class III.—The combined treatment of Classes I and II. This Class III showed markedly better results than observed in either Class I or Class II.

Class IV.—This was comprised of 6 patients presenting the same condition as the 18 patients referred to above. The treatment was the routine dental, local and constitutional plus 0.25 cc of the vaccin at four-day intervals. This dosage was later doubled (0.5 cc). Some of the patients received six, some seven and others eight injections.

Class V.—This was comprised of 6 patients who had never responded to any great degree to persistent dental treatment given at intervals for three years previously. They received the combined local, constitutional and vaccin treatment (0.25 cc at four-day intervals, increasing to 0.5 cc and in 4 cases finally to 1 cc.

The results are presented in tabular form below.

Class II. Positive results	66.66 + per cent
Class III. Positive results	66.66 + "
Class IV. Positive results	83.33 + "
Class V. Positive results	50.00 - "

¹ Dent. Items of Int., 1912, **34**, 122.

² Med. Press and Circ., 1912, **93**, 63, 89.

³ Dent. Cosmos, 1912, **54**, 997.

Cummins¹ reports a number of cases in which vaccins were administered to patients, suffering from pyorrhea alveolaris, for their systemic condition (arthritis, neuritis, indigestion, etc.). The author apparently intends to imply that the administration of the vaccin was useful, but the report is presented in such a confused manner that no conclusion on that point can be reached by the impartial reader.

Betrand and Valadier² applied sensitized vaccins to the treatment of pyorrhea alveolaris. The organisms used were the streptococcus, the pyogenic staphylococci, *M. catarrhalis*, Friedländer's bacillus and the pneumococcus. The *sensitizer* was obtained from immunized animals. The vaccin treatment alone was administered at first until a certain immunity was acquired, when local instrumentation was begun. This procedure reminds one of Goadby's recommendations and is a logical refinement to prevent the establishment of secondary foci or the exacerbations of remote lesions which sometimes follow operative interference with periodontal or periapical infections. After four to six injections it was impossible to reveal by culture from the pockets the presence of the bacterial species isolated before treatment was instituted. These sensitized vaccins gave encouraging results in refractory cases of long standing. The pyorrhea did not return for at least six months (period of observation) after stopping the use of the vaccins.

Medalia³ reported the use of vaccins in 115 cases of pyorrhea alveolaris. Of 14 incipient cases, 13 were cured and the remaining 1 was greatly benefited. Systemic symptoms which were rather prominent were either cured or relieved in all cases while under treatment for their oral condition. The duration of cure varied from at least three months to at least twenty-seven months.

Of 16 moderately advanced cases, 15 were cured and 1 benefited.

Of 85 patients presenting far-advanced pyorrhea alveolaris, 37 were cured, 40 were markedly benefited, 4 unimproved and 3 dropped out. The cures lasted at least from five to thirty-six months. There was noted also a decided amelioration of systemic conditions.

Medalia regards the important infectious agents in pyorrhea to be pneumococci, staphylococci, streptococci and *M. catarrhalis*.

Local treatment and dietary regulation should accompany the administration of vaccins to get the best results.

¹ Jour. Vaccine Therapy, 1913, **2**, 59.

² Compt. rend. Soc. de biol., 1913, **75**, 432.

³ Dent. Cosmos, 1913, **55**, 24, 150.

Laymon¹ reports having used autogenous vaccins in 150 cases of pyorrhea. "The results have been invariably positive, so much so as to warrant the use of vaccins in dentistry."

M. C. Smith² has had uniformly successful results through a period of over three and a half years, from the use of vaccins for pyorrhea. He favors stock vaccins.

T. B. Hartzell³ has used stock and autogenous vaccines in pyorrhea alveolaris. His successes have been fewer than his failures.

Head⁴ observed such an increased improvement locally and systemically in cases of pyorrhea alveolaris and periapical infection following the administration of autogenous vaccins that he could not doubt the great value of this treatment. His experience is based on 40 cases. In about 50 per cent of the cases there was a distinct improvement in the general physical condition. The organisms used included streptococci, staphylococci, *B influenza*, pneumococcus, *M. catarrhalis*, diphtheroids and the Friedländer bacillus. *Streptococcus viridans* was found in about 25 per cent of these 40 cases.

Schuhmann⁵ reports a case of periapical and periodontal suppuration. The patient also had severe rheumatic involvement of left shoulder, elbow and hand. An autogenous vaccin, together with dietary regulation, was administered and relief from the rheumatic condition was gratifying. The oral conditions entirely cleared up. No mention is made of local treatment. A second case of pyorrhea alveolaris, which was very severe and was associated with hepatic enlargement and jaundice, was treated by autogenous vaccin and instrumentation. Two months later there was marked improvement of the oral conditions, and the signs of hepatic disease had disappeared. A third case concerns a patient showing pyorrhea alveolaris, interstitial nephritis and a slight endocarditis. Rest, dietary regulation, local dental treatment and an autogenous vaccin from the oral lesions brought much improvement in two months, both in the general and oral condition.

Davis⁶ reported a few cases of rheumatoid arthritis in which an autogenous, oral, streptococcal vaccin apparently aided in bringing about a favorable outcome. "In a few cases the vaccins appeared to act like a true specific, the patients improving both subjectively and objectively. Many cases were apparently not influenced materially by the vaccin and in some the symptoms appeared to be aggravated."

¹ Dent. Cosmos, 1913, 55, 188.

² Ibid., 189.

³ Ibid., 190.

⁴ Jour. Am. Med. Assn., 1913, 61, 2232.

⁵ Pacific Dent. Gaz., 1914, 22, 792.

⁶ Illinois Med. Jour., 1914, 26, 158.

Hitchens¹ asserts that the results of the use of bacterial vaccins in pyorrhea alveolaris have been so encouraging that before long these agents will be an indispensable adjunct in the treatment of this condition. He does not attempt to determine the responsible microorganism by serological tests (see Goadby and others), but employs a mixed autogenous vaccin composed of all the bacterial species isolated from any *carefully collected* specimen. Each cubic centimeter of the vaccins used by him contains 300,000,000 staphylococci if these organisms be present, and 50,000,000 each of the other bacteria found. Unfortunately no report of concrete clinical experience is given in this paper.

Roddy, Funk and Kramer² reviewed 1496 cases of pyorrhea alveolaris, relative to vaccin therapy. When infectious systemic complications exist an autogenous vaccin is indicated, and even in uncomplicated cases will at times accelerate improvement. Vaccin treatment alone did not especially affect the condition in the mouth. In conjunction with oral hygiene and dental treatment it appeared to exert a beneficial effect in 20 per cent of advanced cases.

Rogers³ refers to 2 cases of diarrhea and pyorrhea alveolaris successfully treated with oral autogenous vaccins.

Womer⁴ briefly summarizes 3 cases of pyorrhea alveolaris in which autogenous vaccins were used. One of the cases failed to respond even after twenty-nine injections and in spite of synchronous dental treatment. The other 2 cases, in respect to which no mention is made relative to local measures, terminated in recovery. One of these 2 cases received four, the other fifteen injections.

Coxon⁵ used autogenous vaccin in the treatment of 38 cases of pyorrhea alveolaris. This method was used in conjunction with instrumentation and other local measures. He is convinced that vaccins constitute a valuable adjunct. In cases of marked suppuration he suggests that it is wiser to try to lessen the inflammation by the use of a vaccin before instituting local measures. This is in line with the recommendations made by Goadby, and Bertrand and Valadier.

Price⁶ makes at least three concrete references to definite cases in which an autogenous vaccin of oral derivation was advantageously employed for the relief of some remote condition: Case No. 1118

¹ Dent. Cosmos, 1915, **57**, 1, 77.

² New York Med. Jour., 1916, **104**, 433.

³ Indian Med. Gaz., 1918, **53**, 121.

⁴ Pennsylvania Med. Jour., December, 1918.

⁵ Brit. Dent. Jour., 1922, **43**, 106.

⁶ Dental Infections and Degenerative Diseases, Clinical, 1923, vol. **2**.

(p. 81), fatigue, heart, neuritis and rheumatism; Case No. 1009 (p. 95), heart and arthritis deformans; Case No. 709 (p. 175), arthritis deformans.

Kritchevsky and Séguin have upheld the view that pyorrhea alveolaris is essentially a spirochetosis.¹ In the 1921 report they refer to a "pyo-iodo vaccin," devised by Weinberg and Séguin.² This is readily prepared. Add about 0.5 cc of pyorrhœic pus to 10 cc of sterile physiological salt solution, so as to have a distinctly opalescent suspension. Then add Gram's liquid until the color is that of light beer (sic!). After ten minutes' contact the microbes are dead and the vaccin is ready for injection. The initial dose is 2 to 4 drops subcutaneously, followed every other day by slightly larger dosages. Following the idea of Goldenberg (see *infra*), Kritchevsky and Séguin have performed intragingival injections of this vaccin and find it better tolerated than such a protein solution as milk. The therapeutic effects they regard in a provisional way as entirely satisfactory.

An entirely new turn in bacterin therapy of pyorrhea alveolaris has been given by Seitz and Goldenberg. Seitz³ used for the antigen various bacteria isolated from many different cases. By prolonged shaking extracts of these organisms were prepared, which were sterilized by filtration through Berkefeld unglazed, porcelain candles. This polyvalent sterile extract was incorporated with the addition of a preservative into a salve. The local application of this product gave encouraging results.

Goldenberg adopted the view of Besredka, that it is logical to induce immunity locally at the affected or susceptible site; in other words, in the cases of gingival infection, in the gingivæ themselves. On account of the structure and architecture of this tissue it is desirable to have an effective dose contained in a minimum volume and also, because this necessitates high concentration, it is desirable to subject the antigen to some detoxicating process. Goldenberg⁴ proposes to do this as follows: He uses exclusively a stock vaccin composed of microbial bodies suspended in an excipient. The bacteria used are streptococci, pneumococci, *M. tetragenus*, *M. catarrhalis* and the Friedländer bacillus freshly isolated from cases of pyorrhea alveolaris. These microbes are mixed in equal parts and the resulting mixture is divided into three portions:

¹ Dent. Cosmos, 1918, **60**, 781-784; Ibid., 1921, **63**, 888-892; Ibid., 1924, **66**, 622-631.

² La Gangrène Gazeuse, Paris, 1918, p. 326.

³ Deutsch. Monatschrft. f. Zahnk., 1921, **39**, 34.

⁴ Compt. rend. Soc. de biol., Paris, 1923, **89**, 65.

Portion 1, consisting of one-half of the mixture, is used to prepare the excipient. The microbes are dissolved in 10 per cent NaOH. After twenty-four hours at 37° C. this is neutralized with HCl.

Portion 2, consisting of one-quarter of the mixture, is heated to 60° to 70° C.

Portion 3, consisting of one-quarter of the mixture, is treated with formol to kill the microbes.

Portions 2 and 3 are suspended in the excipient (Portion 1) and 0.5 per cent phenol is added as a preservative. The final concentration is adjusted so that each cubic centimeter contains 20,000,000,000 (2×10^{10}) of bacteria. The injection is made into the gingivæ. The initial dosage is 0.1 to 0.3 cc (1 to 6 drops). At each new injection the dose is increased by 0.05 cc.

Goldenberg reports briefly that 41 patients with severe pyorrhea alveolaris have been treated in this way. Complete cure was obtained in 38 (92 per cent), while the other 3 showed improvement although the periodontal suppuration persisted.

Frison and de Libouton¹ published just a note relative to Goldenberg's vaccins. They report the almost invariable total and early disappearance of suppuration. Usually no trace of pus exists after the fifth injection. The gums resume their normal color and tonicity. The teeth tighten and the general health shows improvement. Later² de Libouton reports that he had made observations on about 1500 patients, with an average of from 70 to 90 per cent of cures. Briggs,³ although he only presents the concise history of 2 cases, is very favorably impressed with this same method. Duchange⁴ has tried Goldenberg's product in other types of oral infection than pyorrhea, and as a consequence believes it to be a very efficient agent. Jomini⁵ has used Goldenberg's method in a number of cases, and reports on the particulars of 9 of them. His results were uniformly encouraging and satisfactory.

SUMMARY.—In the present state of our knowledge about the use of bacterial vaccins in pyorrhea alveolaris and other oral infections, it would be a mistake to stop here immediately after the presentation of a rather large mass of favorable evidence. It must not be forgotten that these agents are not and have never been widely used and that their employment has probably declined since a time

¹ *l'Odontologie*, 1923, **61**, 841.

² *Dent. Surg.*, May 10, 1924, p. 293.

³ *Brit. Med. Jour.*, February 23, 1924.

⁴ *La Semaine Dentaire*, 1924.

⁵ *Schweiz. Monatschr. f. Zahnheilk.*, 1924, **34**, 339.

which may be dated by the publication of a critique by Merritt.¹ This author admits that there may be a *temporary* improvement in some cases of pyorrhea alveolaris following the use of vaccins; however, he is firmly convinced that these products of themselves neither cure nor permanently improve pyorrhea; that cases which have not yielded to surgical treatment, cannot then be cured by the supplemental use of vaccins; that there is no trustworthy evidence that the combined surgical and vaccin treatment of pyorrhea expedites or increases the percentage of cures. The personal equation may be illustrated in this connection because at least part of Merritt's experience with vaccins was the set of observations reported by Williams (1911) and given in abstract above. This latter author had been very favorably impressed.

In reference to the use of vaccins in the metastatic infections associated with pyorrhea alveolaris, Merritt at this time (1916) was inclined to employ them in certain carefully selected cases. Stillman² supported Merritt's stand. He tried autogenous vaccins in 7 cases of pyorrhea. In none of these was there local evidence of regeneration of the periodontal tissues, cessation of flow of pus or tightening of the teeth. Gordon's experience³ is similar. He regards vaccins as useless in beneficially influencing the local oral condition and as only rarely proving of marked benefit in the systemic disturbances associated with the oral infection. This statement refers to streptococcal autogenous vaccins from oral lesions.

Support of a general nature is given to this attitude of Merritt, Stillman, Gordon and others, by a sentence from Gay's well-known and valuable review of streptococcal infection.⁴ "On the whole we must conclude that there is at present no conclusive evidence of the usefulness of vaccin therapy in streptococcus infection." Besredka⁵ expresses the same opinion. Although Gay and Besredka did not have in mind the use of vaccins in pyorrhea alveolaris, the pertinency of this statement is at once apparent when we recall that streptococci are apparently an all but invariable important factor in periodontal infections, and that these organisms have constituted the basis for most of the vaccins employed. Workers in other fields, *e. g.*, in the treatment of war and industrial wounds, have come to feel the dissatisfaction with bacterins as that expressed

¹ Jour. Allied Dent. Soc., 1916, **11**, 639.

² Ibid., 649.

³ Lancet, 1924, **206**, 1130.

⁴ Jour. Lab. and Clin. Med., 1918, **3**, 721.

⁵ Pansements spécifiques, Ann. de l'Inst. Pasteur, 1924, **38**, 565.

by Merritt and others. Kolmer¹ summarized these experiences as follows: "It is the general consensus of opinion that vaccins had none or but slight direct influence upon the bacterial flora of septic wounds and sinuses; . . ." To appreciate the bearing of this generalization, one need only recall how similar are the conditions obtaining in the pyorrheic pocket to those in "septic wounds and sinuses."

Glynn² recognized that the general experience of those using vaccins for pyorrhea alveolaris, had been one of disappointment, and gives a number of reasons or possible explanations for this failure:

1. That the predisposing cause or causes have not been removed.
2. That the raw surface is continually being reinfected from insufficient attention to local cleanliness.
3. That the vaccin administered is not of the same type as the infecting organism.
4. That the infection is a mixed one, while only an univalent vaccin has been used.
5. That the bacteria producing the infection have been so modified biologically by residence in the patient that satisfactory immunizing response can only be obtained by autogenous vaccins.
6. That the vaccin has been improperly prepared and standardized.
7. That the vaccin has been improperly administered, the doses being too small or too long intervals, . . . or the doses are too large or at too short intervals.
8. That the patient's tissues, for some unknown reason, refuse to respond to the vaccin and produce the necessary antibodies.

The opinion may be hazarded that the use of vaccins in oral infections will never be definitely discarded until or unless vaccin therapy in all bacterial infections is layed aside as useless. The recognition of new microbic species or strains, a revaluation of the relative culpability of the various species in the fauna or flora of the oral lesion, new methods of preparing or of administering the vaccin—any of these factors will require new series of tests to be carried out in reference to the oral disease. Only the accomplishment of this work, which will be largely one of elimination, will permit one to ignore the possibility that a vaccin in a particular case might do some good. This paragraph of course indicates that

¹ Infection, Immunity, etc., 3d ed., 1924, p. 891.

² Med. Press. and Circ., 1912, 93, 63, 89.

research should reserve the right to continue investigations on the employment of vaccins in oral infections.

The bearing of this data upon the problem of the general practitioner or of the periodontist in routine office practice may be seen in the answers to two questions. When account is taken of all the above considerations, does there seem to be a place for bacterial vaccins in the treatment of pyorrhea alveolaris? and, if so, under what conditions may they be employed? The answer to the first question is in the affirmative and finds its justification in the more detailed answer to the second question. In the first place, sole reliance should never, except in exceptional experimental or research investigations, be placed on the vaccin. Bacterial therapy, when used, should be used as an adjunct to various other methods and agencies which are of proved value in relieving the condition. It seems very advisable always to supplement the administration of vaccins by some procedure whose purpose is to increase the exudation into the pyorrhic pocket. This exudate is beneficial by virtue of its content in natural and artificially produced antibodies. MacWatters (1910) to this end used a mouth wash composed of 4 per cent NaCl plus 0.5 per cent sodium citrate, flavored to taste. Kolmer (1924) points out that in the case of the vaccin treatment of septic wounds and sinuses, presenting conditions as we have noted above, analogous to those in the pyorrhic pocket, it is thought desirable to take special measures favoring the access of the antibody-carrying blood plasma to the surfaces of these wounds or sinuses. For example, Wright¹ described a hypertonic saline solution (5 per cent NaCl). The same result may be accomplished by other means, *e. g.*, cupping, Dakin's or Carrell's fluid, hot fomentations, Bier's hyperemia and massage. Some or all of these methods have already been used in the treatment of pyorrhea alveolaris, and it would be easy to adapt them to the present purpose of inducing an increased flow of "opsonized lymph" into the infected gingivæ and pockets.

Indications for Use of Vaccins in Dentistry.—1. When a case of "pyorrhea alveolaris" is refractory and fails to respond to the customary treatment. A number of reports occur in the literature in which in such cases the use of a vaccin apparently induced amelioration and cure of the local oral symptoms: Beebe (1909), Eyre and Payne (1909), Wallis (1911), Williams (1911) and McGehee (1912, Class V). In such cases the possibility must be kept in mind

¹ Lancet, June 23, 1917.

that the refractoriness is due to some systemic trouble, *e. g.*, diabetes mellitus.

There exists a possibility which has not been emphasized by the writers on the subject and which deserves consideration. Our concept of "pyorrhea alveolaris" is that it is primarily a non-infectious condition, which usually becomes secondarily aggravated and complicated by infection. Other things being equal, it is reasonable to expect that the average time during which the teeth could be retained in the mouth in function would be longer where the infectious phase was eliminated or minimized than where this was not the case. From this viewpoint, namely, that of rendering the tissues locally resistant to infection, it is interesting to await the clinical judgment on the attempts to induce local immunity in the gingivæ, either by Goldenberg's method or by the use of specific, bactericidal surgical dressings (see Besredka and Delater, below), or by some other method. Besredka¹ used sterile filtrates of streptococcal or staphylococcal cultures for moistening dressings in a varied number of human conditions with encouraging success. Delater² obtained excellent results in cutaneous lesions by the use of dressings containing staphylococcal and streptococcal vaccins. The benefit observed was attributed to a local immunization at the site of application.

2. Where the operator desires to use all available precautions to ensure the destruction of such bacterial emboli as might be liberated by instrumentation, manipulation of the gingivæ and extraction or to prevent the occurrence of exacerbation of already existing secondary localizations, vaccins afford the most promising agencies at hand. This method is advisable on general principles and not because efficacy has been scientifically established. Local treatment should be undertaken only after the vaccin may be presumed to have increased satisfactorily the resistance. Goadby (1910, 1923), Bertrand and Valadier (1913) and Coxon (1922).

3. Where the patient presents remote or systemic symptoms, reasonably attributable to an oral focus, every effort should be made to eradicate the oral infection. A vaccin may be administered to supplement the effects of this eradication and to assist in eliminating the secondary localizations. Most of the reports on the use of vaccins in cases of pyorrhea alveolaris refer to this phase of the subject. The administration of a vaccin for the relief of some extra-oral condition or symptoms should not be assumed by the dentist

¹ Ann. de l'Inst. Pasteur, 1924, **38**, 565.

² Presse Méd., 1924, **32**, 3.

but should be left to the physician. Billings¹ found in a series of over 500 cases that the final result was quite as satisfactory without as with vaccin in patients suffering with chronic infectious arthritis and acute rheumatism. Patients suffering with chronic *Streptococcus viridans* endocarditis were not benefited by autogenous vaccins and some were probably made distinctly worse when moderately large doses of vaccins were used.

NON-SPECIFIC THERAPY.

It has been found that the parenteral introduction of a great variety of substances can call forth on the part of the macroögranism a variety of reactions or responses, some of which apparently may exert a beneficial influence on the course of an infection. Among such non-specific agents may be mentioned various proteins (normal serum, egg albumen, milk, gelatin), protein-split products, proteoses, pepton, enzymes and cell products, extracts of leukocytes, autolysates of tissues, colloidal metals (gold, silver, platinum, mercury, zinc), hypertonic or hypotonic salt solutions, sugar solutions, distilled water, etc.; the reactions following the injection of such substances include fever, chill, increase in pulse-rate and in the circulatory leukocytes and increased flow of lymph. It is probable that the response to the introduction of every vaccin and immune serum is in part specific and in part non-specific in the sense developed in this paragraph. In some cases the specific effects are the more prominent, in others it is difficult or impossible to separate the specific from the non-specific effects.

¹ Focal Infection, New York, 1917.

CHAPTER XVII.

THE MINIMIZING OF INFECTIOUS DISEASE.

ONE of our large insurance companies in its public advertising recently called attention to the reduction in mortality in certain important causes of death. The reduction observable during the period 1911 to 1923 in the case of tuberculosis was 50.9 per cent; of influenza and pneumonia, 18.8 per cent; of typhoid fever, 77.6 per cent. These instances are not exceptional, rather they represent the general trend. Most infectious diseases have become strikingly less prevalent and less serious during the period of modern medicine, and especially during the past fifty or seventy-five years. Many of the factors contributing to this decline have been consciously conceived and deliberately put into effect by man. Some are the natural sequence of changed economic and social conditions. Finally, some of the factors which have possibly been operative are of quite a different nature, independent of or only incidental to human activities.

Less is known of the group referred to in the last sentence than of the other groups, and consequently we shall take it up first. The disease-producing ability of the infectious microörganism may have become weakened in the course of time. The result would be that fewer people would contract the disease and far fewer would succumb. Syphilis is sometimes advanced as an instance of this. There is evidence that in its recrudescence late in the fifteenth and early sixteenth centuries it ran a far more acute course than is usual at present. The reality of such an attenuation is conceivable but so far its indubitable demonstration has not occurred. As a converse to this suggestion of the attenuation of the microörganism is the possibility that the host species has become in some measure adapted to the parasite. It is also conceivable that the incursions of the disease have more or less succeeded in weeding out susceptible strains from the population. This in a sense would be an elimination of the unfit, and by natural selection the general average of resistance might be raised. We hear often of the "tubercularization" or "syphilization" of the race, and not always is it said with the implication that it is an altogether undesirable goal. The

morbidity and mortality of pneumonia and tuberculosis, for example, is always extremely high in a race or section of the population to which for some generations at least these infections have been unknown.

One of the most peculiar characteristics of some infectious diseases, particularly if they assume an epidemic or pandemic form, is a sort of rough "rhythmicity" in morbidity and mortality.¹ For example, influenza epidemics have been described as occurring on the North American continent in the following years: 1557, 1580, 1647, 1732, 1737, 1760, 1780, 1789, 1805, 1824, 1830, 1836, 1843, 1850, 1860, 1863, 1873, 1874, 1879, 1889, 1891, 1896, 1916, and 1918. The intervals are too variable to permit the discovery of any "law" or to make any probable forecast. During an inter-epidemic period such an infection may be an almost negligible cause of sickness. It is possible that some of our infections show a lowered incidence because they happen to be in such an inter-epidemic phase of their natural history. The causes which determine this "rhythmicity" are almost totally unknown.

In the case of insect-borne diseases as malaria, yellow fever and African sleeping-sickness (trypanosomiasis), conditions altering the distribution or diminishing the numbers of the insect host will affect the human morbidity and mortality of the infection in question.

Personal Hygiene.—In the coming paragraphs attention will be given to the economic, social and medical factors to which may be ascribed a part in the decrease in the frequency of some parasitic diseases. In the first place one of the most obvious contrasts of the present with earlier generations is the fact that the practice of personal cleanliness and hygiene is far more general than ever before. Due to the excesses associated with the Roman baths, bathing itself fell into disrepute with the Fathers of the early Church. The more luxury-loving of their pagan contemporaries glorified the delights of *bagnia*, *vina*, *Venus*. If one is judged by the company one keeps, it is not astonishing that the asceticism and fanaticism of early Christianity was rather inclined at times to regard the neglect of personal cleanliness as a virtue. This neglect continued through many centuries. As late as 1843 Mahomed,² writing of conditions in England, quaintly observed that ". . . a large proportion of the population of this country never submitted themselves to an entire personal ablution in their

¹ Rosenau: *Scient. Monthly*, 1925, 20, 192.

² The Bath, London.

lives, and many an octogenarian has sunk into his grave with the accumulated dirt of eighty years upon his skin” At about this same time the bath tub was an innovation, regarded by some responsible members of the medical profession in this country as dangerous to the health of its user. We do not have to point out that conditions have in this respect changed. Even the Saturday night joke is rare in vaudeville, and the miners of Pennsylvania use their tubs for something else than as a coal bin. As long as current standards of personal cleanliness are maintained infections transmitted by fleas and lice, *e. g.*, bubonic plague and typhus, and many types of obscure skin diseases can never become a serious problem in the United States.

In his comprehensive and critical survey of the Great Plague of London in 1665, Bell¹ estimates in round figures that 100,000 deaths occurred in a population of about 350,000. The active agent in the transmission of this plague is the rat flea. The vast epidemics which more than decimated Europe up into the second half of the seventeenth century (1666) and which devastated parts of Russia and Eastern Europe even after the World War (1914-1918) are unthinkable in this country while present conditions hold.

Fresh Air.—The desirability of fresh air and especially the ventilation of sleeping-quarters are fairly new ideas. The custom in vogue but a generation or two back is reflected in that Christmas verse of childhood popularity:

“I sprang from my bed to see what was the matter.

Away to the window I flew like a flash,

Tore open the shutters and threw up the sash.”

Sunlight.—The prophylactic and therapeutic value of sunlight in the house is appreciated today and people are less afraid of fading carpets or wall-papers than they were in mid-Victorian times. At a still earlier period progressive rulers, in an effort to confine the burden to luxuries, levied taxes on the number of windows in a man's house and thereby encouraged the erection of almost windowless houses. The drop in the morbidity and mortality in tuberculosis is attributable to many factors, among which a keener appreciation of sunlight and fresh air has played no inconsiderable role.

Screening to keep out flies and mosquitoes has really only been widely used since the Spanish-American War (1898); the rat-proofing of buildings and docks as a means to combat bubonic plague is of still later development.

¹ Bell: The Great Plague in London in 1665. London and New York, 1924.

Esthetic considerations have perhaps been the most powerful motivating force in putting the factors mentioned above into operation. It is of course also true that the individual himself, and without the help of his neighbors, can largely realize this goal. The sense of being clean is conducive to a "superiority complex." Sunlight and fresh air are their own reward, quite apart from their other advantages. They are psychic exhilarants. Vermin of all kinds are "Detested, shunned by saint an' sinner," and certainly find less toleration than they did in Burns' day. Alexander is so rare that he is individually recognizable.

Public Health Measures.—The next group of factors we shall term public health measures. The distinction from those involved in the practice of personal cleanliness is not sharp, and is in fact rather arbitrary. Perhaps one difference, such as it is, is this: In the latter group the esthetic motive is strong, while in the case of public health measures one adopts and enforces them because one is intellectually convinced of their utility. This entails a certain education of the medical and non-medical public—an indispensable factor in reducing the incidence of disease. The people *en masse* must be acquainted with the rationale of public health programs before these can be satisfactorily carried through. Perhaps the oldest public health measure is the placing and enforcement of quarantine. The word itself indicates a blanket period of forty days, during which a ship coming from a port where such a disease as plague or cholera was known or suspected to be rampant, was held almost *incommunicando*. The quarantine and medical examination of immigrants are very powerful weapons of defense against the introduction of devastating epidemics. As one instance of this work, we may cite the fact¹ that during the summer of 1911 about 34,000 specimens of the bowel discharges of immigrants and crews from cholera-infected ports were examined bacteriologically. As a result, at New York the cholera vibrio, the cause of Asiatic cholera, was found in 28 persons sick with the disease and, what is of greater importance, also in 27 apparently healthy persons. Seven cases of cholera were detected at other ports by the same methods. As a result of these efforts, not a single case of Asiatic cholera developed in the United States, although steamer connection with infected European ports was not interrupted. Without this unobtrusive protection it is a moral certainty that we would have experienced an at least most disquieting epidemic. The

¹ Anderson: Jour. Am. Med. Assn., 1912, 58, 1748.

severity of quarantine in the commoner diseases, measles, chicken-pox, mumps, whooping cough, scarlet fever, diphtheria, etc., has been much lessened with the growth of exact knowledge about the transmission of infection, and at the present time it is reduced to the minimum compatible with public welfare. In spite of personal inconveniences it should be respected as a matter of personal honor.

The importance of popular education is difficult to overestimate in these matters. Ignorance and indifference are just as real dangers to public and individual health as are the disease-producing bacteria themselves. False modesty, even as late as the adolescence of the present generation, marked all decent discussion of venereal diseases, outside of technical medical circles, as taboo. One of the great services of Mr. Bok is his forcing this problem against much opposition upon the American public through the sheets of the *Ladies' Home Journal*. The integrity and maintenance of the race is absolutely dependent upon the function of reproduction. These infections because of the very nature by which they are transmitted, associated inseparably with one of the two most powerful instincts in all living beings, were striking at the root of our very existence. As long as the rule of silence obtained, to combat their ravages was hopeless. *Tempus fugit* and *tempora mutantur*. It is no longer necessary to bring evidence to the public forum that venereal disease is one of the most serious problems we are facing. That is publicly admitted, even if it still appears at times to be not appreciated by the individual. Not only are extensive programs in operation to reduce this menace, but actual evidence of the success of these programs is available. In Alabama,¹ during a certain period immediately prior to an educational program which gave no attention to disinfectant methods, the admissions of new cases of syphilis numbered 2068; of gonorrhea, 998; of chancroid, 108. During a comparable period immediately after this program had been put into effect the admissions of new cases were respectively 1294, 708 and 34. This means that popular education alone, informing the people of the full dangers and emphasizing continence as the main prophylactic, was followed by a percentage reduction of new cases in the case of syphilis of 37, of gonorrhea of 30 and of chancroid of 70.

To illustrate the value of measures entailing disinfection by the prospective patient himself or at some public or restricted station or clinic, we shall refer to observations made in U. S. Marines at

¹ Welch, S. W.: Southern Med. Jour., 1923, 16, 945.

Honolulu, Hawaii, in 1909-1910.¹ The average venereal infections per 100 men per month without prophylactic disinfection was:

Gonorrhea	6.750
Syphilis	2.850
Chancroid	0.625
Total	10.225

The comparable figures after the introduction of prophylactic disinfection were:

Gonorrhea	3.300
Syphilis	0.750
Chancroid	0.165
Total	4.215

The difference between the incidence before and that after prophylaxis is 6.010. This is an index of the reduction accreditable to prophylactic disinfection. This method was given wide currency in the Army and Navy during the World War and fully proved its worth.

Before leaving the subject of venereal disease mention must be made that the institution of a course of treatment with such a drug as neoarsphenamin soon after a known exposure to syphilis may succeed in completely preventing the appearance of symptoms of this infection.

In the category of necessary public health measures we include the attention given to community water supplies, sewage disposal and the inspection of milk and other foods. In this connection the collection of complete and reliable vital statistics must be mentioned as a most necessary and efficient instrument in decreasing morbidity and mortality. The analysis of these data gives us our problems stated clearly and concretely. We learn in this way where public health measures are needed and also the efficacy of those that are being applied. The prompt reporting to the proper authorities of all communicable diseases is a social duty of paramount importance.

Milk Inspection.—The value of milk inspection is best seen by reference to the deaths from diarrheal diseases in children under five years of age. In 1868 in New York City 36 children died from this cause out of every 1000 living individuals of this age group; in 1872 the rate was 40; in 1876, 27; in 1880, 26; in 1884, 23; in 1888, 21; in 1892, 22, with a gradual decrease from this rate until 1923,

¹ Lane, H. H.: United States Nav. Med. Bull., 1921, 15, 783.

when it was slightly above 2; a drop from 36 to 2+ per 1000 children under the age of five years. Most of this decrease is ascribable to the control of the purity of the milk supply.¹

Water Supply.—The value of attention to water supplies of communities, small or large, is well illustrated by the case of typhoid fever. The vast majority of individuals contracting this infection do so by drinking contaminated water. Bitter experience has taught that all waters in the denser centers of population are to be con-

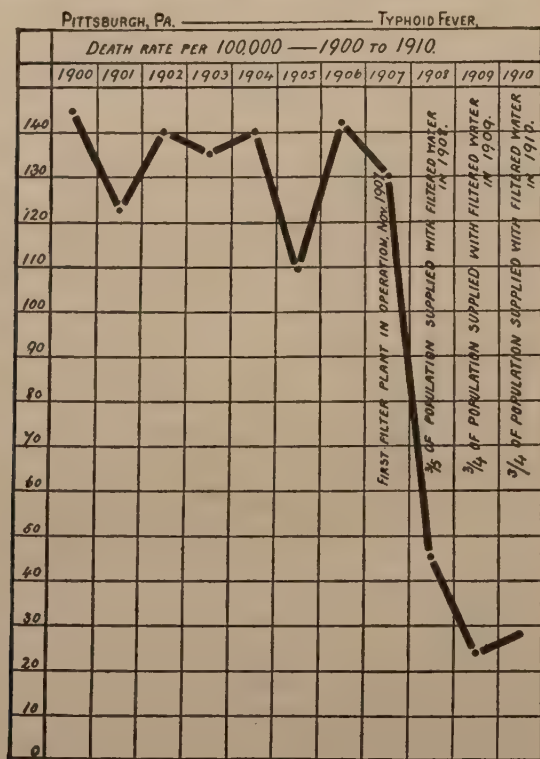


FIG. 70.—(McLaughlin: Communicable Diseases, Harper Brothers.)

sidered polluted unless elaborate and effective measures are carried out to ensure their harmlessness. They are to be considered guilty until proved innocent. The accompanying chart shows the influence of the water supply upon the death-rate of typhoid fever.

Immediately after filtration was put into operation in 1907 the drop in the mortality began. Within three years a new low level had been reached. One hundred and twenty lives for each 100,000

¹ Public Health Reports, United States Treas. Dept., 1924, 39, 528.

of the population were being annually saved. The drop in the morbidity although not shown on the chart was no less striking. In appreciating the benefit from these public health measures we must remember that in addition to the more or less sentimental value of a human life we have vast economic savings. When a man is out of work sick he not only loses his wages but society loses a certain amount of service.

The case of Pittsburgh is not unique. Any of the larger cities of the country could be substituted in its place, to illustrate the point we are making. Before filtration and water purification projects were realized, cities led in high morbidity and mortality from typhoid fever. At the present time, however, due to the effectiveness of these measures, the position is reversed and the rates are higher in the smaller communities and rural districts. A not negligible fraction of the typhoid of cities today is that contracted by their residents during summer vacations in the country.

The good that comes from the purification of water supplies does not stop with the drop in the incidence of typhoid fever or similar "intestinal" diseases. Sedgwick and MacNutt¹ called attention to observation of Mills, Reincke and Hazen, that this public health measure was followed by a general decrease in the mortality of the affected communities. Hazen forcefully stated that "Where one death from typhoid fever has been avoided by the use of better water, a certain number of other deaths, probably two or three, from other causes have been avoided." Sedgwick believed that in at least some instances this estimate was very conservative.

Yellow Fever.—Public health measures are also necessary for the proper control of yellow fever. This problem was forced upon the government of the United States by the Spanish-American War. The cleaning-up of Havana is a matter of history. Later, under more satisfactory conditions, it has been demonstrated that prevention of the breeding of the mosquitoes responsible for the transmission of this infection can completely and rapidly prevent yellow fever. As an instance of this, reference is made to the operations of the International Health Board, Rockefeller Foundation. The chart on p. 304 is taken from the *Seventh Annual Report* (1920), published in 1921.

Malarial Fever.—As malaria is also transmitted by mosquitoes, it may be eradicated by measures directed against these flies. The attendant success is indicated by the accompanying chart. (Fig. 72.)

¹ Jour. Infect. Dis., 1910, 7, 489.

Medical progress in the narrower sense has contributed to the drop in morbidity and mortality in certain infectious diseases, by earlier and more accurate diagnoses and by more appropriate methods of care and treatment. It is impossible to tell with any degree of accuracy how much of the general decrease may be ascribed to these factors. It is easier to demonstrate the effect when some specific measure has come into general use within a very short period of time. Instances of this will follow.

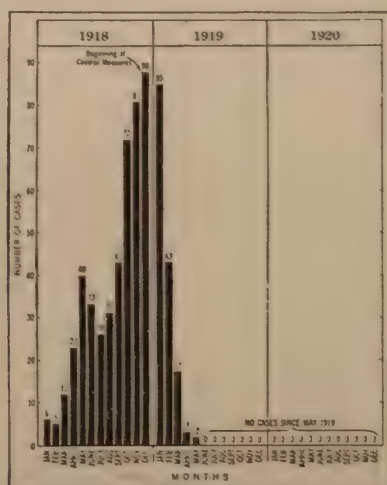


FIG. 71.—Incidence of yellow fever in Guayaquil, Ecuador, 1918-1920. The disease has been completely eradicated from that city as the result of antimosquito measures instituted there in November, 1918. Since May, 1919, there has not been a single case. (Rockefeller Foundation.)

Smallpox.—Some of the most potent factors belong to the category of artificial immunization. The classical example is that of vaccination against smallpox, introduced by Jenner at the end of the eighteenth century. It is hard to realize that for centuries smallpox was a common disease, affecting all social strata. The familiarity which breeds contempt is in no way more obvious than when a mother expresses the hope that her children will have one of the common diseases of childhood. This attitude is based on the moral certainty that at one time or other the infection will be contracted and the sooner the better. Perhaps some idea of the wide occurrence of smallpox may be gained when we know that at one time mothers felt about this disease as they still often feel about measles, mumps and whooping cough. In the *Diary of John Evelyn*, under the date of September 15, 1685, occurs the following

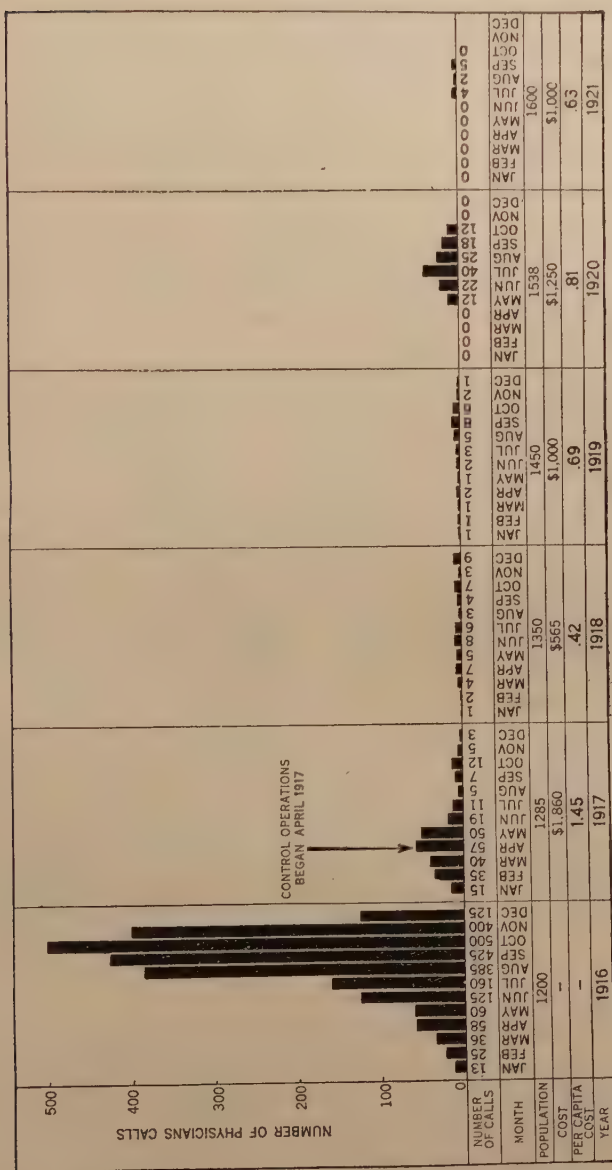


FIG. 72.—Record of malaria incidence for town of Hamburg, Arkansas, which has maintained antimosquito measures for five successive years. The work is now regarded as a regular municipal function. This town and Crossett, Arkansas, were the scenes of the International Health Board's first participation in malaria control by antimosquito measures. (Rockefeller Institute.)

entry: "Her eldest son was now sick of the smallpox, but in a likely way of recovery, and other of her children run about, and among the infected, which she said she let them do on purpose that they might whilst young pass that fatal disease she fancied they were to undergo one time or other, and that this would be the

best" The lady in question moved in the highest circles of English society and had been Maid of Honor to the Queen-Dowager.

Another indication of the frequency of smallpox is given by Voltaire in his *Letters on the English*.¹ The figures in the following quotation are not to be taken as statistically accurate, but Voltaire had a critical mind and we can feel that conditions in 1727-1731 were substantially as represented. "Upon a general calculation three-score persons in every hundred have the smallpox. Of these three-score, twenty die of it in the most favorable season of life,



FIG. 73.—Two brothers and their sister whose father and mother had smallpox. The child in the center was not vaccinated; the other two were. (Welch and Schamberg.)

and as many more wear the disagreeable remains of it as long as they live. Thus a fifth part of mankind either die or are disfigured by this distemper." In the next paragraph he speaks of "Twenty thousand persons whom the smallpox swept away at Paris in 1723"

Compare this with the fact that in the registration area of the United States for 1919 the total absolute number of deaths from smallpox was 358. This means but 1 death for each 250,000 of

¹ Letters concerning the English Nation. Letter XI; Of Inoculation, new edition, London, 1767, p. 62.

the population. The difference between the frequency of small-pox in the time of Evelyn and Voltaire and of the present day may be due to several factors, among which Jennerian vaccination is likely the most important. This measure if rightly applied would be capable of answering for the total reduction.

Rabies.—One of the most dramatic achievements of Pasteur's life affords us another illustration of active immunization, artificially induced. In this case we are concerned with one of the most fatal and horrible of human diseases, rabies or hydrophobia. About 67 per cent of persons bitten by rabid animals and not receiving the Pasteur antirabic treatment, or some modification of it, develop the disease and all cases, once the symptoms appear, end fatally. The mortality equals the morbidity in this infection. During the experiences at the Pasteur Institute of Paris¹ from 1886, when this service was introduced, to 1923 the mortality per 100 persons treated dropped from 0.94 to zero. The mortality of 0.94 means that less than 1 individual in every 100 treated developed the disease and died. The drop from 0.94 to zero is to be accounted for by the spread of education among the laity, bringing them in for treatment soon after being bitten, because the earlier the antirabic vaccination is instituted the more successful it is, and also by minor improvements in the details of the treatment itself.

The saving of life accomplished by the genius of Pasteur in this one disease alone becomes apparent from the following considerations: During the year 1923 at the Pasteur Institute, Paris, 727 individuals received antirabic treatment. Assume that only one-half of these had been bitten by rabid animals and that the other half had presented themselves to be on the safe side. Before 1886, without the treatment, 67 per cent of the former half would have developed the disease and died in the agonies of one of the most horrible of human diseases; *i. e.*, in a single year approximately 243 lives were saved.

Another factor which must not be overlooked when speaking of the drop in the frequency of rabies, is the enactment and enforcement of laws relative to the licensing and muzzling of dogs. Such measures are very successful in lowering the morbidity and mortality. In certain parts of Germany, at least before the World War, this disease had been completely stamped out in this way alone.

¹ Viala: *Ann. de l'Inst. Pasteur*, 1924, **38**, 648.

Typhoid and Paratyphoid Fevers.—As the individual can be protected against smallpox and rabies by vaccination, so he can also be protected in a similar fashion against typhoid and the paratyphoid fever. In these instances the protective agency is supplementary to measures ensuring the purification of water supplies and the purity of foods. The need of such supplementary protection is obvious in an army, especially when in the field. It is also useful for those anticipating travel or sojourn in countries or districts which fail to give attention to their water supplies. Each season more individuals who are merely going on a brief summer's vacation take advantage of it. The vicissitudes and uncertainties of an auto tour for a holiday make antityphoid vaccination particularly desirable.

The following table from Rosenau¹ shows the value of this means of active immunization.

TYPHOID FEVER, 1901-1912, FOR THE WHOLE ARMY, AT HOME
AND ABROAD—OFFICERS AND ENLISTED MEN.

Year.	Mean strength.	Cases.		Deaths.			Occurring among those who were vaccinated.	
		No.	Ratios per 1000 of mean strength.	No.	Ratios per 1000 of mean strength.	Per cent of total cases.	Cases.	Deaths.
1901	81,885	552	6.74	74	0.88	13.0		
1902	80,778	565	6.99	69	0.85	12.2		
1903	67,643	348	5.14	30	0.44	8.6		
1904	67,311	293	4.35	23	0.33	7.8		
1905	65,688	206	3.14	20	0.30	9.7		
1906	65,159	373	5.72	18	0.27	4.8		
1907	62,523	237	3.79	19	0.30	8.0		
1908	74,692	239	3.20	24	0.31	10.0		
1909 ²	84,077	282	3.35	22	0.26	7.8	1	0
1910 ²	81,434	198	2.43	14	0.17	7.1	7	0
1911 ²	82,802	70	0.85	8	0.10	11.4	11	1
1912	88,478	27	0.31	4	0.044	14.8	8	0
1913	80,766	4	0.04	...	...	...	2	0
1914	87,228	7	0.07	3	0.03	42.8	1	0

The decrease in the morbidity and mortality after 1910 is striking. Particular attention is directed to the two right-hand columns. Of a total of 588 cases occurring during the six years, 1909-1914,

¹ Preventive Medicine and Hygiene, New York, 2d ed., 1916, p. 107, Table III.

² Typhoid vaccination was voluntary during 1909 and 1910, and until September 30, 1911, when it was made compulsory for officers and men.

only 30 developed among those who had been vaccinated. Of 51 deaths occurring during that same period only 1 occurred among those who had been vaccinated. This latter consideration points out another advantage in antityphoid vaccination. Not only is the morbidity and mortality lower, but such cases as do occur usually run a very mild course. This is apparent in the following table adapted from Kolle and Hetsch.¹

Clinical course.	Percentage of 906 cases occurring in the uninoculated.	Percentage of 371 cases occurring in the inoculated (vaccinated).
Mild	36.55	50.13
Moderately severe	24.85	25.88
Severe	25.80	17.52
Fatal	12.80	6.47

Until the World War disease had always been more fatal to armies than direct combat. In the Spanish-American War (Reed, Vaughn and Shakespeare) out of a force of 107,973 there were 20,738 cases of typhoid (and paratyphoid) fever with 1580 deaths. By contrast, in the U. S. Army during 1917 and 1918, with an enlistment far over 1,000,000 and antityphoid vaccination almost universal, the total absolute number of cases was only 1065; *i. e.*, fewer cases from among over 1,000,000 vaccinated than there were deaths from among slightly over 100,000 unvaccinated. In the U. S. Army, from September 1, 1917, to May 2, 1919, there were only 213 deaths from typhoid fever. As Russel has pointed out, if the Civil War death-rate had obtained during the World War then there would have occurred 51,133 deaths; if the Spanish-American War death-rate had obtained during the World War then there would have occurred 68,164 deaths. The saving of life, largely by antityphoid vaccination, balanced all the deaths from combat suffered by the United States in the World War.

Diphtheria.—The latest major achievement of preventive medicine has been the discovery of a simple and safe method, practicable in every sense of the word, which if taken advantage of will render diphtheria as rare as smallpox now is. This method consists in the injection under the skin of a minute quantity of the peculiar poison of the diphtheria bacillus, neutralized by the long-known diphtheria antitoxin. The recipient of this injection often is aware of no sense of discomfort. The reaction is most trivial and far less obvious than even that following vaccination against smallpox. The efficacy of this toxin-antitoxin vaccination against diphtheria will become apparent from what follows. In

¹ Experimentelle Bakteriologie, 1916, 4te Aufl., Bd. I., p. 325.

1923 Park and his co-workers¹ reported on their work in New York City. They selected two groups of children, 90,000 to each group. The first group consisted of those who were found to be naturally resistant to diphtheria plus those who had been artificially protected by the toxin-antitoxin mixture. In the course of time 14 cases of clinical diphtheria were reported from this group. The second group was chosen as a control. It included among its 90,000 both naturally resistant and those susceptible to diphtheria. None of them had received the artificial protection afforded by the toxin-antitoxin vaccine. In this group 56 cases of clinical diphtheria were reported, *i. e.*, exactly four times as many as occurred in the first group. This comparison does not serve to bring out the full value of the method. To do this the second or control group should have been limited to those who had been proved to be susceptible and who had not received any artificial protection. During the two years preceding this report the number of cases of diphtheria in New York City had diminished by 20 per cent, and the death-rate had decreased from 20 to 16 per 100,000 of the population. Park ascribes these results to the use of the toxin-antitoxin mixture (active immunization) and to the spreading of information relative to the use of diphtheria antitoxin (passive immunization).

Other communities have had a similar experience. This method of active immunization has been tested out with particular thoroughness at Auburn, N. Y.² Fifty-eight per cent of the school children had been tested for their susceptibility, and if found susceptible then received the toxin-antitoxin protection. From this group 7 cases of diphtheria, with 1 death, were reported. On careful examination most of these 7 cases proved to be suffering not from diphtheria but from another type of sore-throat. The confusion arose because they happened at the same time to be diphtheria "carriers." The single case of death in this, the larger group of children, occurred in a boy who had remained for some unknown reason persistently susceptible in spite of the fact that earnest efforts had been made to render him resistant.

By way of contrast we have a second or control group, comprised of 42 per cent of the school children of Auburn. None of them had been treated by any method for increasing their resistance to diphtheria. As in the New York City control group, this group at Auburn contained the naturally resistant as well as the sus-

¹ Park, Schroder and Zingher: *Am. Jour. Pub. Health*, 1923.

² Sears: *Am. Jour. Pub. Health*, 1924, **14**, 210.

ceptible. From it were reported 83 cases, with 14 deaths from diphtheria.

At Seneca Falls, N. Y., the average annual number of cases of diphtheria had been for five years 22. Then the susceptible children had been actively immunized. In the succeeding year only 1 case developed.

At East Syracuse, N. Y., for eighteen months preceding the administration of toxin-antitoxin to susceptible children, 144 cases, with 8 deaths, had occurred. In the next five months not a single case was reported.

In Syracuse, N. Y., following the active immunization of 81 per cent of the school children, the cases and deaths decreased more than 50 per cent in one year.

It becomes apparent that in this toxin-antitoxin agency we have a most effective means for the eradication of diphtheria. It is also probable, although as yet unconfirmed by broad clinical experience, that similar protection can be developed for all those infections which owe their distinctive characteristics to a type of bacterial poison which is technically known as a toxin. In this category are tetanus, gas gangrene and scarlet fever. Concerted effort is being made at the present time to make this possibility a reality in the case of scarlet fever. Confidence is felt that in the near future this children's disease will follow diphtheria to become along with smallpox chiefly of historic interest.

Nurses and dental hygienists, particularly those working in schools and children's asylums or homes, should be tested for their susceptibility to diphtheria and scarlet fever. If found susceptible (Schick positive or Dick positive) they should be actively immunized against these infections.

Active Immunization.—The protection afforded against typhoid fever, smallpox and diphtheria, which has been described above, is technically known as active immunization. This means that the treatment used stimulates the tissues of the recipient's body to a particular form of activity. There are actively produced ways and means of destroying or nullifying the germ in question. Some of the protection is due to an increased quantity of certain substances in the circulating blood. The process is directly comparable to that which occurs when a person in the natural course of events recovers from one of these diseases. Common experience is that he is less likely, usually far less likely, to contract that same infection a second time. The protection may not be as great or

as lasting when it is secured artificially as when it comes naturally as a sequel to recovery.

Passive Immunization.—The idea presented itself to some of the early students of bacteriology: If we have protective substances in high concentration in the blood of one animal or individual, why cannot we take these ready-made substances, transfer them to a susceptible individual or to one suffering from the disease, and thereby increase the resistance of this second individual. The idea proved to be grounded in fact. This type of resistance or protection is technically known as *passive* immunization, because the

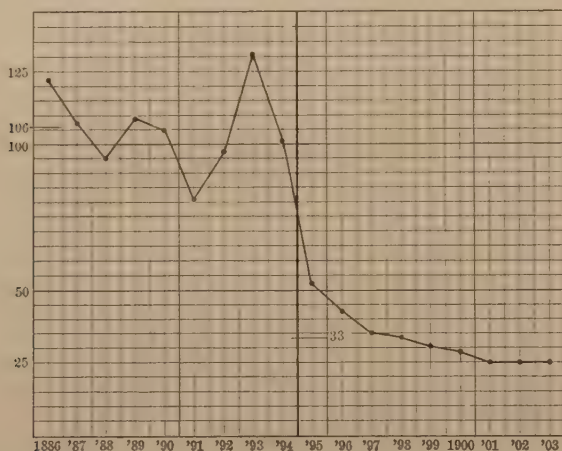


FIG. 74.—The numbers at the left indicate the mortality from diphtheria per 100,000 of population in German cities with population over 15,000. The numbers along the base line indicate the years from 1886 to 1903 inclusive. The period to the left of the vertical line (1886-1894) was before the general application of the antidiphtheritic serum; to the left of this line (1895-1903) is represented the period after its general application. The average mortality per annum for the first nine years was 106; for the second nine years, 33. (From Jaeger.)

benefit accruing to the second individual comes not from his own efforts, but he is merely the passive recipient of the products of another's efforts. The difference between *active* and *passive* immunization is like the difference between a fortune built by oneself and that inherited from some one else. The first is apt to last the longer and give the greater satisfaction.

The classical and most important instance of passive immunization is furnished by the use of antitoxin for the prevention and in the treatment of diphtheria. The accompanying chart (Fig. 74) shows the remarkable drop in the death-rate following the general utilization of diphtheria antitoxin. The same result has been

universally experienced. Before the introduction of this agency the yearly death-rate from diphtheria in Massachusetts was 74.4 per 100,000. In 1921 this rate was 15.5. In Boston before 1894 the annual absolute number of deaths ranged from 400 to 850. By 1920 it had dropped to 120—this notwithstanding the increase in population. In Philadelphia the diphtheria mortality per 100,000 of the population for the five years preceding the use of antitoxin was as follows:

[illegible]

In the five years following its general adoption the figures are as follows:

1906	37.78
1907	34.60
1908	33.35
1909	33.60
1910	31.70

Remarkable as is the success indicated by these statistics, the value of antitoxin becomes more apparent when it is given early in the course of the disease. A fatal result is almost precluded by its administration on the first day of the attack, but its effectiveness is diminished rapidly by each day of delay. This becomes obvious from the accompanying chart (Fig. 75).

Passive immunization is also useful in protecting individuals who have been exposed to infection. Diphtheria antitoxin is frequently given to the other children of a family when one of them has come down with diphtheria. Many cases and many deaths have unquestionably been prevented by this procedure, but it is difficult to estimate with any accuracy the actual numbers. In Baltimore, for example, Doull¹ found that according to the records of 508 consecutive cases of diphtheria 10 per cent of the family contacts ten years old or younger who were not given prophylactic antitoxin subsequently developed diphtheria, mostly within thirty days. Of the children of the same age group who were given prophylactic antitoxin only 1.2 per cent were attacked. Unless special contraindications exist, the use of prophylactic antitoxin in young children who have been in close contact with a case of diphtheria, is clearly not only justified but demanded.

¹ Pub. Health Repts. United States Treas. Dept., 1924, **39**, 283.

Some light on the value of this preventive or prophylactic use of passive immunization is also yielded by the experience during the World War with tetanus. The age-long cultivation of the soil in Belgium and northern France had brought about a relatively high concentration of tetanus bacilli. Lacerated wounds contaminated with this soil frequently afforded the portal for these microorganisms. The number of cases developing per 1000 wounded rose, as can be seen in the diagram (Fig. 76) in the early months of the World War to most lamentable heights. Then, at once following the injection of tetanus antitoxin routinely into all wounded, the incidence dropped to an average of less than 1 case in each 1000 wounded. This same method is generally applied in certain types of industrial accidents.

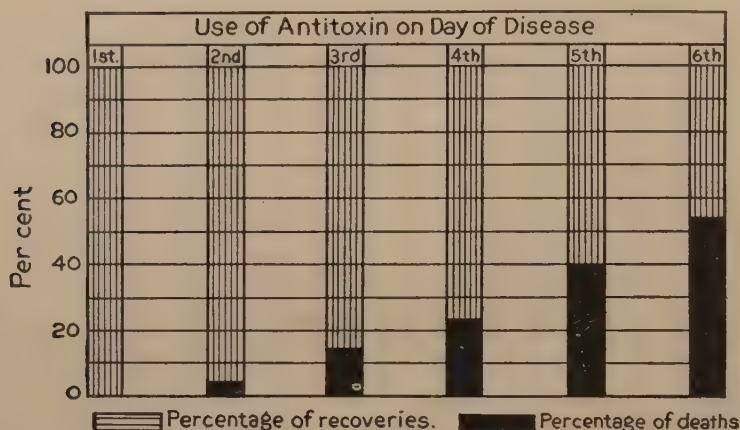


FIG. 75.—Percentages of recoveries from diphtheria when antitoxin is administered on various days in the course of the disease. Prepared by Schereschewsky on data of Kolle and Hetsch. (Moore, Public Health, Harper Brothers.)

Of greater practical interest in civil life, is the passive immunization which has been perfected in this country in very recent years against scarlet fever. The pioneer work was largely done by Dick and Dick, of Chicago, by Dochez, of New York City, and by Blake, of New Haven, Conn. The administration of the antitoxic serum is followed by the disappearance of the rash, a drop in temperature rapidly to normal and the patient himself is conscious of a marked improvement. This same serum can be used to protect individuals who have come in contact with the patient.

Certain cases of pneumonia are amenable to serum treatment. This has been introduced since the World War, and although the

results are less satisfactory than in the case of diphtheria, tetanus and scarlet fever, nevertheless there is no question but that it is of definite practical value in some cases.¹

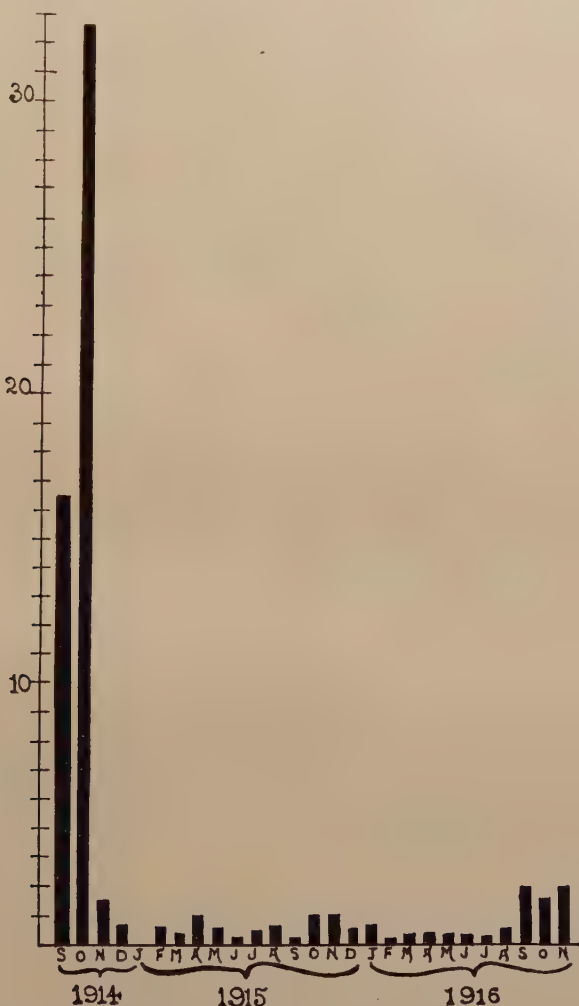


FIG. 76.—Incidence of cases of tetanus in British wounded per thousand (Fitzgerald).

The above paragraphs have shown that many communicable diseases are becoming less frequent and less fatal. Some of the factors which are at work in bringing about this decrease have been considered. Gratifying and hopeful as the situation is, in com-

¹ Wadsworth, A. K.: *Am. Jour. Hyg.*, 1924, **4**, 119.

parison with conditions of the not long-distant past, much remains to be done when what could be done is taken as the ideal. In the conflict with the causes of disease one can never rest on his oars. This is lamentably apparent in communities where long freedom from the worst aspects of smallpox or rabies or diphtheria has dulled the old horror once connected by these infections. Smallpox vaccination is not enforced, dogs are allowed to run unchained and unmuzzled, diphtheria antitoxin is not given until several days after the child has fallen sick. In each case in a few months or a few years the negligence, the false sense of security must be paid for with economic loss, physical and mental suffering and death. The human race must be irrepressibly optimistic so quickly does it forget its lessons. Eternal vigilance is the price of health no less than of political liberty.

Few can be unaware that much more remains to be done in decreasing the toll of disease. What has gone before is in the nature of a promise. One of the most significant generalizations in the history of civilization has been formulated by Dr. Hermann M. Biggs: "*Public health is purchasable, within certain limitations a community can determine its own death-rate.*" This is well known to those officially concerned with the condition of public health—but the realization of its implication calls for the intelligent participation and coöperation of every individual citizen.

ORAL HYGIENE.

Although the immediate objective of dentistry be the maintenance and restoration of the function of the masticatory apparatus, an ulterior motive—and possibly one of greater social significance is afforded by the fact that the realization of the primary objective benefits the human organism as a whole by minifying diseases of extra-oral parts.

Elsewhere in this volume it has been pointed out that postoperative pneumonias are less likely to occur in individuals with healthy mouths than in those with neglected mouths. It would seem reasonable to extend this same idea in an effort to reduce the terminal pneumonias, particularly among the aged in whom oral conditions are often deplorable. Similarly if postoperative parotitis be an ascending infection along Stenson's duct, it seems obvious that as a prophylactic measure preoperative attention to the oral condition is indicated.

Likewise a badly infected mouth will favor the occurrence of

the complicating secondary infections, pyogenic and fusospirillary, in patients suffering from pulmonary tuberculosis. Systematic, thorough oral hygiene in these individuals would to some extent lighten the burden under which they are laboring. Oral infection should be radically removed and scrupulously avoided particularly in this class of patients. It seems at present unwise in these cases to temporize with pulpless teeth, irrespective of their history or roentgenographical appearance. As a corollary it may be pointed out that oral disease favors the oral localization of tuberculosis secondary to pulmonary involvement. Secondary oral localizations are almost or quite unknown in well-cared-for mouths. Similarly the mercurial treatment of syphilis can be pushed more intensively and effectively where the oral cavity is healthy than where it is harboring infection on a large scale.

Steadman,¹ on a number of occasions, has maintained that oral infection predisposes to cancer of the alimentary tract and associated parts, and has offered evidence that neglected oral sepsis shortens the duration of life of patients affected with alimentary carcinoma.

The significance of oral focal infection, its occasional relation to arthritis, endocarditis, gastric ulcer, iritis, the secondary anemias, etc., will be considered in a separate chapter in Part III.

Finally, there is considerable evidence indicating that the practice of oral hygiene, using this term in its widest meaning, renders the child less susceptible to the so-called children's diseases. This statement is made with a full appreciation of the difficulty of definitely establishing its correctness. Fones,² in a survey of five years of mouth hygiene in the public schools of Bridgeport, Conn., calls attention to the reduction in the mortality from three of the most serious infections of childhood:

	1914, per cent.	1918, per cent.
Diphtheria	36.6	18.7
Measles	20.0	4.1
Scarlet fever	14.1	0.5

In the summary he explicitly states that "Mouth hygiene is to be a very powerful factor in the restriction of communicable and infectious diseases in childhood."

¹ Proc. Roy. Soc. Med., Odont. Sect., 1914, 7, 37; Dent. Cosmos, 1914, 56, 789.

² Dent. Cosmos, July, 1919.

PART III.

SPECIAL INFECTIONS OF THE ORAL CAVITY.

CHAPTER XVIII.

THE STREPTOCOCCUS-PNEUMOCOCCUS GROUP.

At the present time it seems justifiable to regard the streptococci as the single most important group of bacteria from the dental standpoint. Most of the significance attached to oral infectious foci is dependent upon the presence of these microorganisms in almost all periapical and chronic periodontal lesions. Biologically the streptococci and pneumococci are apparently close relatives. In fact reports of transmutations among various members of this group are not uncommon. Rosenow¹ reported strains of hemolytic streptococci becoming under various conditions changed into organisms having the characteristics of *S. viridans*, of pneumococci and of *S. mucosus*. Strains of *S. viridans* have, it is claimed, been converted into pneumococci, into *S. mucosus* and into hemolytic streptococci; similarly strains identified on isolation as pneumococci have undergone transformations justifying their identification as hemolytic streptococci and *S. viridans*. *S. mucosus* has become first a hemolytic streptococcus and then a *S. viridans*. Rosenow was aware that such results might be interpreted on the assumption that the original cultures were mixed, and that the varying conditions would now favor the predominance of one type and now of another. To anticipate these objections some of his work was done with cultures developed from a single bacterial cell (Barber technic). Pulvermacher and Schnitzer² and Freund³ reported the change of a *S. viridans* into a hemolytic type. Pruska⁴ has reported the transformation of a hemolytic streptococcus into a non-hemolytic

¹ Jour. Infect. Dis., 1914, **14**, 1.

² München. med. Wehnschr., 1923, **70**, 866.

³ Deutsch. med. Wehnschr., 1923, **49**, 1146.

⁴ Cas. lék. ces., per Centralbl. f. Bakteriöl., Abt., I, Ref., 1924, **77**, 208.

type and *vice versa*. Many observers on the other hand have not encountered such transmutations,¹ which on the whole are regarded today with some skepticism.

By pointing out their similarities and by contrasting their differences we shall gain a better understanding of the characteristics of the streptococci and pneumococci. Both belong to the spheroidal bacteria (Coccaceæ). The pneumococci are typically grouped in pairs, while the streptococci occur in chains. This grouping is generally valid, although exceptions are frequent (Fig. 77). Pneumococci, particularly Type III (see *infra*), may occur in short chains, and streptococci often, especially after cultivation in artificial media, appear in pairs. Streptococci in sections of pneumonic



FIG. 77.—Pneumococcus from bouillon culture, resembling streptococcus. (Park and Williams.)

lung may appear in pairs. The modification from the spheroidal form may afford a most important point of differentiation. The typical pneumococcus when seen in sections of tissues or in stained smears directly from the pathological exudate has a peculiar lance-shaped form. Each cell of the pair is somewhat flattened where it is in contact with the other cell, while opposite this contact surface the outline of each member of the pair is contoured to a point. On the other hand, each member of a streptococcal chain is usually quite regularly circular in outline, although where reproduction has been rapid the chain appears to be formed of a number of paired units, *i. e.*, the unit of the chain seems to be a

¹ Howell: Jour. Infect. Dis., 1924, **34**, 122; Schottmueller, München. med. Wehnschr., 1924, **71**, 1009.

diplococcus, a pair of spheroidal bacteria, rather than a single spheroidal cell. Such "diplococci" do not resemble the pneumococci, but each member of the pair presents a hemispheroidal form with the flattened surfaces approximating.

Pneumococci when appropriately stained in tissue sections or in smears from pathological exudates or from culture media containing native proteins are surrounded by an envelope or capsule. Streptococci are not encapsulated. The so-called *S. mucosus*, which presents a capsule, probably is the Type III pneumococcus.

All pneumococci and all streptococci pathogenic for man are Gram-positive.

The majority of pneumococcal strains ferment the polysaccharid, inulin with the formation of acid, a property not possessed by the majority of streptococcal strains.

A more reliable distinction than is afforded by the fermentation of inulin, is furnished by what is designated as "bile solubility." When a small quantity of bile or of bile salts is added to a culture of pneumococci the organisms readily undergo dissolution. If the suspension was turbid the turbidity disappears; no sediment forms; pneumococci cannot culturally be recovered from the liquid and stained smears show no bacteria. On the contrary, streptococci not only are not dissolved by bile or bile salts but continue to grow in media containing these constituents. This difference, though on the whole dependable, does not afford an infallible criterion. Some pneumococci are bile-insoluble and some streptococci are bile-soluble. As a rule, the bile-soluble pneumococci are more virulent for, at least, the white mouse than are the bile-insoluble pneumococci.¹ Avery, Chickering, Cole and Dochez² believe that the phenomenon of insolubility must be comparatively rare, since among several hundred strains of pneumococcus isolated by them from lobar pneumonia none has failed to be dissolved by bile.

The technic for bile solubility given by Cotoni, Truche and Raphael (p. 48) is simple and reliable:

I. Three drops of a mixture of equal parts of rabbit bile and glycerin is added to 1 cc of a twenty-four-hour old liquid, serum-free culture of the microorganism. Clarification, when it occurs, will be complete at room temperature within a few minutes. The substitution of a 5 per cent solution of sodium taurocholate in physiological salt solution for the bile-glycerin mixture is permissible.

¹ Cotoni, Truche and Raphael: Pneumocoques et affections pneumococciques, 1922. Monographie. L'Institut Pasteur.

² Monograph No. 7, Rockefeller Inst. Med. Res., 1917, p. 14.

Ox bile is less reliable than rabbit bile for this purpose. When substituted it should be tested out with known bile-soluble and bile-insoluble cultures for controls. Sodium taurocholate or sodium glycocholate (10 per cent in physiological salt solution) in the proportion of 0.2 to 0.1 volume to actively growing broth cultures may be used.¹

II. The technic described by Avery, *et al.*, follows: Fresh ox bile, obtained directly from the slaughter house, is autoclaved for twenty minutes, at 15- pounds' pressure. The bile is filtered to remove the precipitate formed on heating and again autoclaved. The sterile bile is now ready for use. For routine purposes 0.2 to 0.1 volume of whole bile is added to the plain broth culture.

The virulency of pneumococci is, as a rule, far higher than that of streptococci for the white mouse. Glynn and Digby² summarize their experience with "mass" inoculations: "A sputum or saliva containing pneumococci was more pathogenic to mice, judging by their mortality and duration of life after inoculation, than one containing streptococci . . ." Cotoni, Truche and Raphael³ report death of the mouse following the intramuscular injection of 0.00000000001 cc of a pneumococcal culture. The determination of virulency of these organisms should be made as early as possible as it is a property which usually is rapidly lost on artificial cultivation. Comparative virulency tests, however, as in the case of inulin fermentation and bile-solubility, do not by themselves permit the separation of a streptococcus from a pneumococcus. Strains of the former organism, possessing the higher degree of pathogenicity, are not at all uncommon.

Serological tests, *i. e.*, precipitin and agglutinin reactions, will permit a clear distinction to be drawn between the streptococci and at least certain pneumococcal strains. This phase will be discussed further on.

Although the pneumococcus is a little the more exacting in respect to its growth requirements than the streptococcus, the ordinary media, used routinely in every laboratory, *e. g.*, agar-agar, gelatin, broth, potato and litmus milk, are less useful than the rather special tests which have just been enumerated. Avery, Chickering, Cole and Dochez⁴ sum up their experiences as follows: "Pneumococcus is bile-soluble, possesses a capsule, ferments inulin, is extremely pathogenic for mice and on blood agar forms a small

¹ Avery, Chickering, Cole and Dochez: Monograph No. 7, Rockefeller Inst. Med. Res., 1917, p. 14.

² Med. Res. Council, Special Report Series, London, 1923, No. 79.

³ Loc. cit., p. 30.

⁴ Loc. cit., p. 16.

moist, flat, ringed, checker-like colony with a greenish zone of methemoglobin about it. Moreover . . . the various types of pneumococci react specifically with their homologous immune sera. Streptococcus, on the other hand, is not bile-soluble, does not ferment inulin, is less virulent for mice; on blood media the colony is more opaque and raised, drier and more coarsely granular, without the surface markings or ringed topography characteristic of pneumococcus, and is surrounded by a zone of either hemolysis or green pigmentation."

The appearance of the colony on properly made blood-agar plates serves to distinguish very sharply between the pneumococcus and some streptococci. This difference will be better understood further on when the streptococci are considered in some detail. For the present it will suffice to note that the pneumococcus colony on the blood-agar plate is not confusable with the colonies of the hemolytic and "indifferent" streptococci, but does show a marked resemblance to the colony of the "viridans" or "alpha" streptococci (Plate III, Fig. 3). Schottmueller¹ observes that the pneumococcus on blood-agar plates "forms an intensive dark green pigment;" Brown² explicitly states that the pneumococcus colony presents the same appearance as that described by him for the alpha type of streptococcus.

THE PNEUMOCOCCI.

These organisms were independently and almost at the same time discovered by Pasteur³ and Sternberg⁴ from the saliva. Their pathogenic significance was not recognized until the studies of Fraenkel⁵ and Weichselbaum,⁶ who demonstrated their relationship to human pneumonia. From this time on for over twenty years it was believed that a pneumococcus was just a pneumococcus. The work of Neufeld and Haendel,⁷ however, showed that these organisms differed among themselves as far as their immunological reactions were concerned. This line of research was assumed on a large scale at the Rockefeller Institute, with the result that Avery, Chickering, Cole and Dochez⁸ divided the strains isolated by them into four groups or types, which are universally known as Types

¹ München. med. Wehnschr., 1903, No. 20 and No. 21.

² Monograph No. 9, Rockefeller Inst. Med. Res., 1919, p. 19.

³ Bull. de l'Acad. méd., 1881, 2, 94.

⁴ Bull. Nat. Board of Health, 1880-1881, 2, 781.

⁵ Ztschr. f. klin. Med., 1886, 10, 526.

⁶ Med. Jahrb. N. F., 1886, 1, 483.

⁷ Arb. a. d. kaiserl. Gsndtsamte, 1910, 34, 293,

⁸ Loc. cit.

I, II, III and IV. The principal means of distinguishing these types are the precipitin and agglutinin reactions. Differences of relative frequency and pathogenicity of these types become apparent, but only when dealing with large numbers of strains. The freshly isolated individual strain can only be correctly placed by immunological "typing." Types I, II and III constitute relatively well-defined groups or entities, although Type II presents a number of atypical sub-groups.¹

On the other hand, Type IV does not represent any such biological entity. Its nature is perhaps best described by considering it a miscellaneous pigeon-hole in which are filed such pneumococci as do not belong to the three well-defined types, I, II and III. Abstractly considered, *i. e.*, apart from clinical medicine, there are many races or serological types of pneumococci. Three of these repeatedly occur in a large percentage of human cases of pneumonia, and are consequently set apart and dignified with the designation of Type I, Type II and Type III. All the other serological races occur with far lesser frequency, and are consequently lumped together into a very heterogenous group, the so-called Type IV. The pneumococci habitually found in the mouth usually belong to this group, and the infections caused by them usually run a mild course.

The pneumococcus is responsible for about 90 per cent of all cases of lobar pneumonia. The relative frequency and virulency of the four types in this country is given by the following table from Avery, Chickering, Cole and Dochez:²

Type of pneumococcus.	Incidence, per cent.	Mortality, per cent.
I	33	25
II	31	32
III	12	45
IV	24	16

A therapeutic serum against Type I infection which has some beneficial effect has been prepared. Wadsworth³ has reviewed the recently published reports and 445 additional cases. He concludes

¹ Stillman: (Jour. Exper. Med., March, 1919) studied 204 strains of pneumococci, all of which showed atypical agglutination in Type II serum. Of these strains 100 were obtained from normal mouths. In classifying these strains agglutination and absorption reactions were employed. On the basis of specific agglutination in monovalent rabbit sera, the 204 strains were classified into twelve groups (IIa to IIm). These sub-groups have an incidence of 11 per cent in lobar pneumonia and of 18 per cent in normal mouths. Certain groups (IIb, IIc, IIf and IIm) occur in normal mouths. Sub-groups IIa and IIh are met with largely in connection with disease.

² Loc. cit., p. 33.

³ Am. Jour. Hyg., 1924, 4, 119.

that when all factors have been taken into consideration there would seem to be no question but that antipneumococcus serum of high potency, when promptly administered in adequate dosage, is of definite practical value in the treatment of Type I infections of pneumonia.

Until the studies of the workers at the Rockefeller Institute it was believed that pneumococcal pneumonia was an autoinfection. That is, pneumococci were often (40 per cent) present in mouths of healthy individuals, and with lowered resistance these directly invaded the lungs. The following table adapted from Avery *et al.*,¹ shows why this view is untenable.

DISTRIBUTION OF DIFFERENT TYPES OF PNEUMOCOCCUS.

Type.	Percentage incidence in normal mouths.	Percentage incidence in lobar pneumonia.
I	0.8	33.3
II	0.0	29.3
IIa	0.8	1.3
IIb	5.8	0.9
IIx	11.6	2.0
III	28.1	13.0
IV	52.9	20.3

About 66 per cent of the cases of pneumococcal lobar pneumonia are caused by organisms which are not commonly inhabitants of "normal" mouths; therefore, it is likely that in many of such cases the pneumococci, Type I or II, were acquired by direct or indirect contact with another individual carrying respectively Type I or II pneumococcus.

The so-called "Type IV" pneumococcus is of some interest to the dentist as a common inhabitant of the human mouth.

Miller² injected 1 or 2 drops of the mixed saliva of apparently healthy individuals intraperitoneally into 111 mice; 48 were dead within four days. It is very likely that in at least some of these Type IV pneumococcus was the responsible agent. Although representatives of this group were found in less than 25 per cent of all unselected cases of pneumococcal pneumonia, they occur in a far higher percentage among the postoperative pneumonias. Whipple³ reported that of 22 cases of surgical pneumonias which had been "typed" 77 per cent showed Type IV and only 16 per cent belonged to the other three types. In a later study⁴ he reported

¹ Loc. cit., p. 90.

² The Human Mouth as a Focus of Infection, Dent. Cosmos, September, October and November, 1891.

³ Med. Rec., 1916, 89, 581.

⁴ Surg., Gynec. and Obst., 1918, 26, 29.

the analysis of 97 cases, showing the following incidence of pneumococcal types: Type I, 4; Types II and III, 5 each; Type IV, 46. Cleveland¹ continued Whipple's studies and concluded that of the organisms present the pneumococcus group IV holds the preëminent place, being present in 32 per cent of patients before operation and in 57 per cent after operation in cases developing postoperative pneumonitis. Joslin and Gage² in a small series found the type of organism in one-half of the cases in which this was determined to be Type IV; 3 cases proved to be of Type III and 4 to be of Type II.

These facts suggest that the invading microorganisms in postoperative pneumonia are derived from the patient's own upper respiratory tract and mouth. Whipple,³ in fact, found in many of these cases the pneumococcus IV isolated from both preoperative and postoperative sputum to be the same by positive agglutination tests with the patient's serum. The view that postoperative pneumonia is largely an aspiratory infection is also supported by the fact that its incidence is lowered among those who have had their mouths freed from gross sepsis previous to general narcosis.

Pathogenicity of Pneumococci.—In addition to pneumonia they "may give rise to inflammation of the upper respiratory tract and the accessory sinuses of the nose. Infection of the middle ear and invasion of the meninges may also be due to pneumococcus. Primary peritonitis of pneumococcus origin may occur, especially in young children. Focal infections, as empyema, peritonitis, pericarditis, endocarditis, arthritis and lung abscess due to pneumococci may occur as sequelæ of pneumonia."

THE STREPTOCOCCI.

The status of the classification of the streptococci is not, at least from the standpoint of clinical medicine, as satisfactory as that of the classification of the pneumococci. The changes induced by the developing colony in a blood-agar plate seem to be of fundamental differential significance. Schottmueller⁴ first pointed this out. Brown⁵ has studied this phase of the subject very thoroughly and

¹ Surg., Gynec. and Obst., 1919, 28, 282.

² Med. Clin. North America, 1918, 2, 469.

³ Loc. cit., 1918.

⁴ Die Artunterscheidung der fuer den Menschen pathogenen Streptokokken durch Blutagar, München. med. Wehnschr., 1903, No. 20, p. 849; No. 21, p. 909.

⁵ The Use of Blood Agar for the Study of Streptococci, Monograph No. 9, Rockefeller Inst. Med. Res., 1919.

PLATE III



Streptococcal colonies in 5 per cent blood-agar; 16 mm. obj., 3 oc. 1, alpha type; 2, alpha prime type; 3, pneumococcus; 4, beta type; 5, gamma type; 6, delta type. The central dark area represents the bacterial colony. The outer, yellowish zone represents the unchanged blood-agar. In this zone the granules and clumps represent the erythrocytes, while the pale, yellowish background is due to the hemoglobin which has laked from the cells. Note hemolysis by alpha, alpha prime, and beta streptococci and by the pneumococcus. Note the essential similarity of the effects of the alpha streptococcus and the pneumococcus. Note the methemoglobinization of the erythrocytes by alpha and delta streptococci and by the pneumococcus. Note the disappearance of the laked hemoglobin but the persistence and methemoglobinization of the erythrocytes in the case of the delta streptococcus.

has given the best descriptions of the well-recognized groups of streptococcus distinguishable on blood agar. The identification of Brown's types requires the study of the *deep* colony with a low-power objective (not greater than 16 mm.). The medium should be practically free from dextrose. The nutrient agar should have been liquefied and cooled to 45° to 50° C. To this is added about 5 per cent by volume of sterile, defibrinated horse, rabbit or human blood, without preservative. Usually 0.6 cc of the blood added to 12 cc of the agar will give enough medium to furnish the optimum depth when poured into a Petri plate of 9 cm. inside diameter. The inoculation should not be heavy. It is easiest to read results when a plate presents from five to thirty colonies. Further, Brown's method does not permit of final reading until after forty-eight hours' incubation at 37° C. plus forty-eight hours' refrigeration.

The *alpha* (α) (Plate III, Fig. 1), after forty-eight hours' incubation, macroscopically examined, exhibits small, biconvex, greenish, deep colonies, surrounded by a zone in which the blood corpuscles are distinctly greenish. Under the low power of the microscope the greenish zone is less apparent, but toward its periphery there may be suggestion of hemolysis.

After forty-eight hours' refrigeration microscopical examination of the deep colonies shows them to be surrounded by double zones: (1) A distinct inner collection of greenish or brownish, non-hemolyzed erythrocytes next to the colony, and (2) an outer more or less hemolyzed ring. The hemoglobin of the greenish or brownish, non-hemolyzed corpuscles has been transformed into methemoglobin. Alternate incubation and refrigeration will produce further concentric zones of methemoglobinized corpuscles and hemolysis.

The *alpha* type of streptococcus corresponds with Schottmueller's *Streptococcus mitior seu viridans*.

Type *beta* (β) (Plate III, Fig. 4) is characterized by a deep colony, surrounded by a perfectly clear, colorless zone of hemolysis. There is no trace of discoloration and when viewed microscopically no corpuscles are seen in the medium surrounding the colony. The colony itself is grayish by transmitted or reflected light. Refrigeration for forty-eight hours brings no change. Brown's type *beta* streptococcus is to be identified with Schottmueller's *S. longus pathog. seu erysipelatis*.

Type *gamma* (γ) (Plate III, Fig. 5) is characterized by a deep colony which develops without any perceptible hemolysis or deep

discoloration of the surrounding medium during incubation or refrigeration.

Type *alpha prime* (α^1) (Plate III, Fig. 2), according to Brown, can scarcely be called anything but a hemolytic streptococcus. Hemolysis is distinctly present, without apparent discoloration of the persisting erythrocytes. To the naked eye colonies of the *alpha prima* type appear somewhat hazy or poorly defined within the hemolyzed zone. Under the microscope the reason for this haziness is apparent: A few erythrocytes are seen to have remained throughout the zone, but are most numerous next to the colony. Alternate incubation and refrigeration does not determine the appearance of multiple zones of concentric rings.

A fifth, or *delta* (δ) (Plate III, Fig. 6), type has been described by Bryant.¹ It is characterized by methemoglobinization of the erythrocytes without any hemolysis. Such hemoglobin as has leaked into the medium is rendered colorless. Deep colonies after forty-eight hours' incubation appear macroscopically irregular-shaped, olive-green in color and about 0.75 mm. in diameter. Each colony is surrounded by a pale green zone slightly narrower than the diameter of the colony. The ill-defined outer margin of this greenish zone is not surrounded by a clear colorless (hemolyzed) zone, but merges directly into the unchanged blood agar beyond. Refrigeration brings no change. Microscopically the blood cells immediately around the colony appear as minute, pale, gray-brown dots, or, when seen in clumps, as slightly darker gray-brown masses. There is no apparent decrease in the number of erythrocytes, but they lie in an absolutely clear, colorless background. The outer margin of this zone is very ill defined, and merges gradually into the unchanged medium where the agar is tinted a distinct pink from the leaked hemoglobin and the blood cells have retained their normal tone, the clumps especially being seen as definitely red masses.

Summary of Types of Streptococcal Colonies on Blood Agar.—When deep colonies are studied, especially microscopically, according to Brown's specifications, the following types have been distinguishable:

1. *Type alpha*: A zone immediately around the colony consisting of methemoglobinized erythrocytes and an outer zone of hemolysis. This peripheral zone is emphasized by refrigeration.
2. *Type beta*: The colony surrounded by a clear, colorless zone in which hemolysis has been complete; not changed by refrigeration.

¹ Jour. Bacteriol., January, 1925.

3. *Type gamma*: The developing colony has induced no discernible changes in the environing medium, *i. e.*, neither hemolysis or methemoglobin formation.

4. *Type alpha prime*: The colony is surrounded by an indistinct zone of hemolysis in which still persist some erythrocytes whose hemoglobin has not been changed into methemoglobin.

5. *Type delta*: The colony is surrounded by a zone of methemoglobinized cells. No hemolysis, *i. e.*, disintegration of the erythrocyte, occurs.

Reported observations of the transmutation of hemolytic streptococci into non-hemolytic strains and *vice versa*, as well as the transmutation of pneumococci into non-hemolytic streptococci and *vice versa*, have been made not infrequently. The difficulties of establishing such transmutation beyond doubt are very great. Until further evidence is available it is probably wisest to suspend judgment. The weight of analogy is against the reality of such transmutations. Typhoid bacilli apparently remain typhoid bacilli and do not become or are not derivable from colon bacilli. Typhoid bacilli and the closely related paratyphoids seem to maintain their own individualities. The three well-known strains of tubercle bacilli, the human, bovine and avian, are not mutually interchangeable.¹

Nomenclature.—The nomenclature of the streptococci in its unsatisfactoriness and confusion reflects the uncertainties of their classification. Many of the terms are only of historical interest, and their retention in current bacteriological, medical and dental literature only unnecessarily complicates the situation. It seems desirable, however, to attempt to place some of the more familiar names in Brown's classification. It is easier to decide which strains, races, types or species of streptococci belong to the beta type. This includes *S. pyogenes*, *S. erysipelatos*, *S. longus*, *S. scarlatinæ*, *S. anginosus*, *S. hemolyticus*, *S. hemolysans*, *S. vulgaris*, *S. infrequens* (Holman), *S. equi* (Schuetz), *S. subacidus* (Holman) and *S. alactosus* (Smith and Brown).

The alpha type certainly includes organisms referred to by such names as *S. mitior*, *S. viridans*, *S. brevis*, *S. salivarius*, *S. fecalis*, *S. mitis*, *S. equinus* and *S. ignavus*. Brown has identified as of his gamma type, *S. saprophyticus* (Mandelbaum) and *S. anhemolyticus vulgaris* (Zangemeister). Organisms going by the names of

¹ Cobbett: The Causes of Tuberculosis, Cambridge Univ. Press, 1917.

S. anhemolyticus or *S. non-hemolyticus* might be referred to any type but beta, although possibly alpha prime would also be excluded.

Subdivisions of Brown's Types.—The alpha, alpha prime, beta, gamma and delta types are not homogeneous groups but are composed of many sub-groups, the problem of whose taxonomic rank we are not even ready to approach. Until rather recently the criteria which have been employed in establishing these sub-groups have been the fermentative power of the organism in question, tested on a great variety of sugars, polysaccharids, glucosids and polyatomic alcohols. Rather elaborate systems of streptococcal classification have been based on the presence or absence of fermentative power toward a number of such fermentable compounds. The better known of these classifications are those of Gordon,¹ of Andrewes and Horder,² of Holman³ and of Blake.⁴

The present consensus of opinion on the value of classification based on fermentative powers seems to be that it is not clinically useful. In brief pathogenicity and fermentative powers do not appear to be correlative variations or characteristics among the streptococci. However, as a matter of record and to facilitate the dentist placing a freshly isolated streptococcus in these more or less classical systems, the following tables are given:

TYPES OF STREPTOCOCCI; TOTAL OF 1200 STRAINS.⁵

Name.	Milk clot.	Neutral red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Saltin.	Coniferin.	Mannite.	Growth on gelatin at 20° C.	Morphology.	Pathogenicity for mice.
A. <i>S. equinus</i> . . .	—	—	#	—	—	—	#	#	—	—	Medius	—
B. <i>S. mitis</i> . . .	—	#	+	+	—	—	#	#	—	+	Brevis	—
C. <i>S. pyogenes</i> . . .	—	—	+	+	—	—	#	—	—	+	Longus	+
D. <i>S. salivarius</i> . . .	+	#	+	+	#	—	—	—	—	#	Brevis	—
E. <i>S. anginosus</i> . . .	+	#	+	+	#	—	—	—	—	#	Longus	+
F. <i>S. fecalis</i> . . .	+	+	+	+	—	—	+	+	+	+	Brevis	—
G. <i>Pneumococcus</i> . . .	#	—	+	+	+	#	—	—	—	—	Brevis	+

¹ A Ready Method of Differentiating Streptococci and Some Results Already Obtained by Its Application, *Lancet*, 1905, ii, 1400.

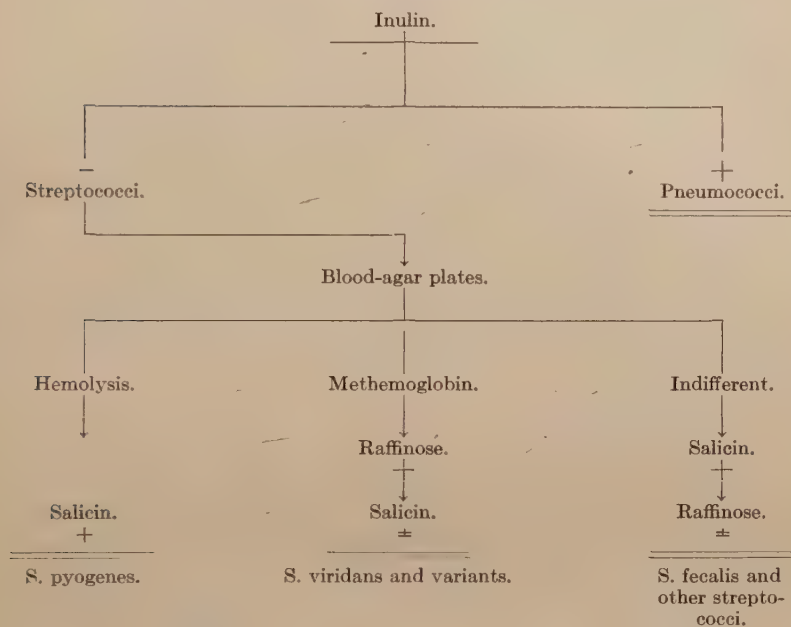
² A Study of the Streptococci Pathogenic for Man, *Lancet*, 1906, ii, 708, 775, 852.

³ Classification of Streptococci, *Jour. Med. Res.*, 1916, **34**, 377.

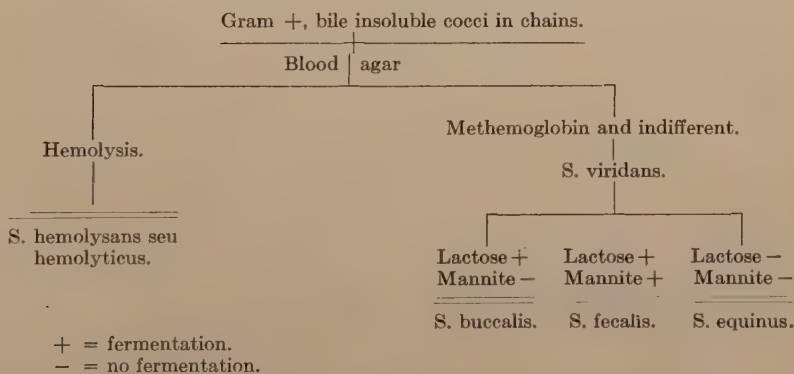
⁴ *Jour. Med. Res.*, 1917, **36**, 99.

⁵ Andrewes and Horder: *Lancet*, 1906, ii, 708, 775, 852.

TYPES OF STREPTOCOCCI; 263 STRAINS OF STREPTOCOCCI AND PNEUMOCOCCI.¹



CLASSIFICATION OF STREPTOCOCCI.²

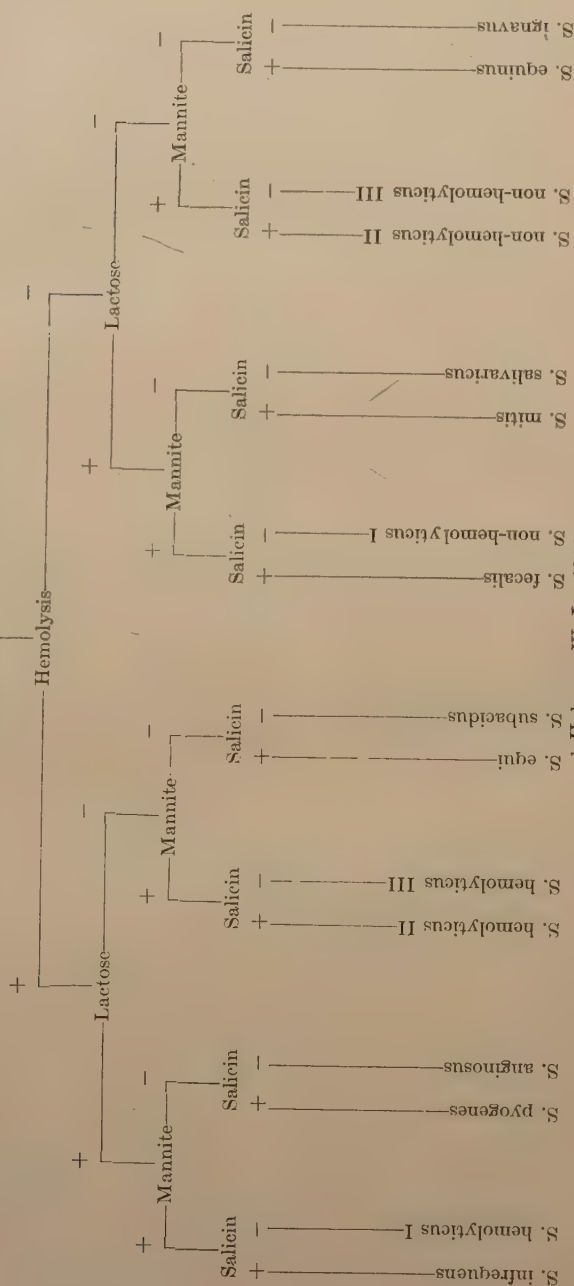


¹ Lyal: Jour. Med. Res., 1914, **30**, 487, 515.

² Blake: Jour. Med. Res., 1917, **36**, 116.

CLASSIFICATION OF STREPTOCOCCI.¹

Streptococci, Gram-positive cocci in chains; no capsules.

¹ Holman, W. L.; 1916

The attempt to establish classifications on immunological relationships, which has on the whole proved so satisfactory in the case of the pneumococci, has been among the streptococci at least in part encouraging. Tunnici¹ has established on the basis of opsonic and agglutinin absorption tests that the hemolytic streptococci associated with scarlet fever form a distinct serological group. This fact has proved of the greatest value in the work of Dick and Dick, of Dochez and of Blake to whom we owe a therapeutic anti-scarlatinal serum which promises high effectiveness. Later Tunnici² presented evidence confirming the reality of serological unity among the streptococci of scarlatina and extended this demonstration of serological unity to the streptococci of erysipelas. Stevens and Dochez³ by means of agglutination and agglutination-absorption tests confirmed the work of Tunnici¹ in respect to the serological unity of scarlatinal streptococci. It is also likely that a similar serologically homologous group is afforded by the streptococci of epidemic milk-borne sore-throat. Smith and Brown⁴ found the streptococci from any given epidemic a homogeneous group, but the streptococci from different epidemics were not necessarily serologically related.

Attempts to define serological groups of clinical import among the alpha streptococci has as yet not yielded the success obtained among the beta streptococci. Krumweide and Valentine⁵ regard the *viridans* strains as heterogeneous on the basis of agglutination tests. Kinsella and Swift⁶ studied alpha type streptococci from acute rheumatic fever and bacterial endocarditis. Two groups of streptococci on the basis of complement-fixation reactions were distinguishable, corresponding to the two clinical entities. Katharine Howell⁷ found less specific fixations among the *viridans* than among the hemolytic group, but observed that strains from specific disease entities tend to fall together.

Rosenow⁸ and Tunnici⁹ found that many of the alpha type streptococci isolated during the pandemic of influenza fell into a specific group as determined by agglutination reactions.

¹ Specific Nature of the Hemolytic Streptococcus of Scarlet Fever, Jour. Am. Med. Assn., 1920, **74**, 1386.

² Jour. Am. Med. Assn., 1920, **75**, 1339. ³ Jour. Exper. Med., 1924, **40**, 253.

⁴ Jour. Med. Res., 1914-1915, **31**, 455.

⁵ A Study of the Agglutination and Cultural Relationship of Members of the So-called Streptococcus Viridans Group, Jour. Infect. Dis., 1916, **19**, 760.

⁶ A Classification of Non-hemolytic Streptococci, Jour. Exper. Med., 1917, **25**, 877.

⁷ Complement Fixation of Streptococci, Jour. Infect. Dis., 1918, **22**, 230.

⁸ The Occurrence of a Pandemic Strain of Streptococcus during the Pandemic of Influenza, Jour. Am. Med. Assn., 1919, **72**, 1608.

⁹ Observations on Green Producing Cocci of Influenza, Jour. Infect. Dis., **26**, 405.

A valuable summary of the work of others and a presentation of extensive original studies is given by Alice Evans.¹ It appears to be clearly established that the alpha type constitutes a heterogeneous group (see also Hitchcock²). In this respect, it resembles the Type IV pneumococcus. Practically nothing is known concerning the serology of the other (gamma, alpha prime, delta) streptococcal types.

One of the most urgent problems facing the dentist or physician in the presence of oral lesions from which streptococci have been cultivated is to determine their actual or potential culpability. Is the strain now, or will it in the future be, responsible for an arthritis, an endocarditis, a cholecystitis or other lesion is a question which cannot honestly be ignored. Until it is definitely established and generally accepted that all streptococci from oral lesions are potentially dangerous, the question of the extraction or retention of a pulpless tooth will recur. Some discredit, though unjustifiable, has come to the doctrine of focal infection because the removal of a pulpless, periapically infected tooth has not brought relief from some more or less remote condition. These instances emphasize the need of some reliable prognostic method. Efforts in this direction have been made by Potter, McNeil and Bradbury,³ and by Zabriskie⁴ who used the complement-fixation test. The fact that alpha streptococci preponderate in oral lesion and that alpha streptococci are very heterogeneous immunologically renders the problem very difficult unless it can be shown that the oral alpha streptococci are serologically homologous, or that the alpha streptococci found in the various secondary localizations are severally homologous. If it were demonstrated, for example, that most streptococci from gastric ulcers belonged to any peculiar serological group and if a streptococcus having the reactions of this group were found in a periapical infection, the dentist or physician would find it easy to come to a definite conclusion as to the fate of the tooth.

The desirability of some such method of facilitating prognosis in oral infection is obvious, but in spite of the work referred to, practically no progress has been made toward its development. The available data are too few to permit of or to justify general or routine clinical application.

¹ A Study of the Alpha Type of Streptococcus from a Variety of Sources, Hyg. Lab. Bull. No. 134, Treas. Dept., United States Public Health Service, 1923.

² Jour. Exper. Med., 1924, **40**, 575.

³ Med. Rec., 1918, **93**, 135.

⁴ New Jersey Dent. Jour., April, 1919,

Hemolysis and Virulency.—Many efforts have been made to correlate hemolytic power with virulency or pathogenicity. While diversity of opinion still exists and while hemolytic power *ipso facto* does not invariably indicate high pathogenicity, there can be no question but that the streptococci associated with acute and urgent, often generalized, diseases, such as puerperal septicemia, erysipelas, epidemic sore throat, Ludwig's angina and scarlet fever, belong to the truly hemolytic or beta type. On the other hand the streptococci associated with the more chronic localized, less obviously or dramatically serious disorders, such as we find in the focal infections, endocarditis, arthritis, gastric ulcer etc., do not usually belong to the beta type, but rather to the alpha type.

The streptococci isolated from oral lesions are usually of the alpha type. Gilmer and Moody¹ found in their study of alveolar abscesses and infected root ends many graded variations from a hemolytic streptococcus with a wide zone of hemolysis in acute abscesses to a *Streptococcus viridans* in the chronic. They encountered just once, as the predominating organism, what they regarded to be a *Streptococcus mucosus*. Hartzell and Henrici² cultured 162 cases of oral infections and 150 of these obtained streptococci. In almost every case blood-agar plates were made and in all but 2 of these alpha type organisms were obtained. These 2 are referable to the gamma type. No hemolytic or beta streptococci were obtained. Moody³ secured 49 cultures from 55 patients suffering from alveolar abscess. In nearly every instance a pure culture of *Streptococcus viridans* (alpha type) was obtained. Occasionally he encountered stray colonies of staphylococci and hemolytic streptococci.

In the routine examination of cultures from root canals at the Evans Institute, the alpha type streptococci far outnumber all other types combined. The gamma and delta come next in frequency, and the beta last. We never have seen a representative of the alpha prime type. Such beta as we found have not been associated with acute abscesses (see Gilmer and Moody *supra*).

The type of streptococcus isolated from the periapical tissues in the course of root-canal treatment has not been considered in its prognostic bearings. Concrete evidence is not available and

¹ A Study of the Bacteriology of Alveolar Abscesses and Infected Root Canals, Jour. Am. Med. Assn., 1914, **63**, 2033.

² A Study of Streptococci from Pyorrhea Alveolaris and from Apical Abscesses, Jour. Am. Med. Assn., 1915, **64**, 1055.

³ Pathogenicity of Streptococci from Alveolar Abscesses, Jour. Infect. Dis., 1916, **19**, 515.

until it is, it would seem discrete for the clinician to shape his course by the following considerations. As already pointed out, the beta or hemolytic streptococci are usually associated with the more acute, urgently serious diseases while the alpha type is associated with the more chronic, localized, less obviously or immediately serious diseases. These facts have suggested that hemolysis and virulence were directly related. McLeod¹ has studied this question very carefully. He found that sixteen authors expressed disbelief in any direct relation between hemolysis and virulence.² Six authors were neutral. Nine authors believed in a definite and direct relation between these two properties, and in seven additional publications there is indirect evidence seeming to confirm the latter view. McLeod from his own observations concludes that the degree of hemolysin production in the animal body is closely related to virulence. Subject to individual exceptions, this conclusion would find general acceptance today. In a series of papers Ando in collaboration with others³ has studied the relation of streptococcal hemolysis to virulence. He recognizes two subdivisions of the beta type, on plates made according to Brown's directions; a typical and an atypical. The diameter of the zone of hemolysis around the colony of the atypical beta type may be greater or less than that around the typical beta colony. The line of demarcation between the hemolyzed zone and the surrounding unchanged erythrocytes is sharp in the case of the typical and ill-defined in the case of the atypical colonies. Further, the hemolytic zone around the typical beta colonies is always free from persisting erythrocytes while this is not always so in the zone around the atypical colonies. These authors (Ando and collaborators, Ito and Chen) are convinced that their typical beta streptococci are pathogenic while their atypical beta streptococci are either saprophytes or only slightly pathogenic. In other words completeness and definiteness of hemolysis are associated with virulence.

When these facts are taken in connection with the inherent anatomical obstacles to removing periapical infection *via* the

¹ Criteria of Virulence Amongst Streptococci with Some Remarks on Streptococcal Leukocidin, Jour. Path. and Bacteriol, 1918, **19**, 392.

² Stevens, Brady and West (Jour. Exper. Med., February 1, 1921) found that an increase of virulence (*S. pyogenes* of Holman) for any one species of animal did not produce greater concentration of hemolysin than the original strain. The possibility that the observed lack of correspondence between hemolysis and virulence might be due to the more effective adaptation of the original strain to the conditions of artificial cultivation, would have to be eliminated before these results could be accepted as definitely proving the absence of any correlation between hemolysin production and virulence.

³ Kitasato Arch. Exper. Med., 1923, vol. **6**.

root canals, the practitioner would be justified if, when he encountered a beta streptococcus, especially one of Ando's typical betas, he gave up root-canal medication and resorted at once to root amputation if indicated or to extraction.

Elective Localization of Streptococci.—Rosenow has observed that a streptococcus isolated from a lesion in a given organ or part, when injected into a rabbit or dog, tends to become localized in the organ or part corresponding to its source. This is spoken of as elective localization. Its occurrence is not invariable and only becomes obvious when rather large series of experimental animals are considered in their totality. Its successful demonstration requires rather close adherence to Rosenow's technic, for which one is referred to his original reports.¹ The examination of the following table which was adapted by Gay² from a table published by Rosenow in the first two of the above references, will serve to show definitely what is meant by elective localization and will also serve to present some of the evidence in favor of this concept.

Original strain.	Per cent lesions with non-specific strains (animals, 833; strains, 220).	Per cent lesions with specific strains.
Appendix	5	68
Ulcer, stomach	20	60
Cholecystitis	11	80
Rheumatic fever	27 joint	66
	14 endocardium	46
	2 pericardium	27
	10 myocardium	44
Erythema nodosum	2 skin	90
Herpes zoster	2 skin	70
Mumps	0 parotid	73
Myositis	10	35
Endocarditis	14	84

Many bacteriologists do not accept this doctrine of Rosenow as valid. For example, Detweiler and Maitland³ decided that the location of lesions in animals seemed to bear no relation to the origin of the organism or to the lesions produced by it in the patient from whom the strain was obtained. Few have succeeded in repeating Rosenow's results; but even admitting the facts to be essentially as he presents them, they would interpret the results in different ways, for example, on the assumption that the dominant factor in the localization was not resident in the parasite but in the host's

¹ Jour. Am. Med. Assn., 1915, **65**, 1687; Dent. Cosmos, 1917, **59**, 485; Jour. Dent. Res., 1919, **1**, 205.

² Jour. Lab. and Clin. Med., 1917, **3**, 721, Table II, p. 742.

³ Jour. Exper. Med., January 1, 1917.

organ or part. Haden¹ has presented rather striking confirmation of the actuality of elective localization, whatever be the interpretation: 68.2 per cent of 66 rabbits inoculated with the cultures obtained from the oral lesions of 15 patients suffering from metastatic eye infection developed lesions in the eye; 14.8 per cent of 169 rabbits inoculated with cultures from patients other than those suffering from eye disease developed lesions of the eye.

Elective localization, where it can be demonstrated, affords dramatic evidence in favor of the doctrine of focal infection; illustrating as it does a causal connection between the primary focus and the secondary localization.

¹ Arch. Int. Med., 1923, **32**, 828.

CHAPTER XIX.

THE SPIROCHETES OF THE MOUTH.

THE chief habitat of spirochetes in man is the oral cavity. Because of the technical difficulties of isolating these organisms there exists great uncertainty upon the questions of: (1) How many different kinds are there; (2) what are their taxonomic relationships, and (3) their pathogenicity.

Historical.—Much of the literature, which is fairly voluminous, is valueless and serves only to confuse. In 1875 Cohn¹ remarks that recently he had found spirochetes in the mucoid deposits on the teeth. Two years later Koch² records that spirochetes are constantly present in the human mouth, that they closely resemble the organism of recurrent fever, and that they are very variable in regard to their length and thickness. This latter observation led Koch to suggest that these varieties perhaps represented different developmental stages. Trevisan³ included the organisms described by Cohn and by Koch in a single species, "*Spirochæte Cohnii*." Some time later the spirochætes of the mouth were divided into two morphologic types: (1) The larger, with relatively few, loose, irregular coils, the *Spirochæta buccalis*, Steinberg and (2) the smaller, whose coils were more numerous, tighter and regular, the *Spirochæta dentium*, Miller. In passing, the frequently encountered statements that the larger form was named by Cohn in 1875 and the smaller by Koch in 1877 are incorrect. Arndt⁴ introduces the term "*Spirochæte denticola*" and applies it apparently indiscriminately to all the spirochetes of the mouth. As late as 1892 in his classical work Miller⁵ in some places uses the terms "*Spirochæte dentium*" and "*Spirochæte denticola*" synonymously, to designate all the oral spirochetes. He does however voice the opinion (p. 68) that in the human mouth there may be two different spirochetes. The only oral spirochete mentioned by Goadby

¹ Beitr. z. Biol. d. Pflanzen, 1875, Bd. I., H. 2., S. 180.

² Cohn's Beitr. z. Biol. d. Pflanzen, 1877, Bd. II., H. 3., S. 399.

³ Rendi conti. Reale Istituto Lombardo di Scienze e Lettere ed Arte. IV, Ser. II. 1879, 12, 149.

⁴ Arch. f. path. Anat., 1880, 79, 76.

⁵ Die Mikroorganismen der Mundhöhle, Leipzig, 2d ed., 1892.

(*Mycology of the Mouth*, 1903) is the "*Spirochæta dentium*," occurring in fine irregular threads, $0.1\ \mu$ wide and 5 to $7\ \mu$ long. He hazards the opinion that it is identical with the *Spirillum sputigenum*. This is certainly not true for the latter organism grows on ordinary media.

In 1984, Plaut¹ incidentally mentioned some cases of a diphtheroid angina with "bacilli of Miller" and spirochetes. The "bacilli of Miller" may as well refer to the *Spirillum sputigenum* and the comma bacillus of Miller as to the fusiform bacillus. The invariable presence of spirochetes in these diphtheroid anginas was pointed out by Vincent,² at least by 1904. The interest which these observations had already aroused in the pathogenicity of these microorganisms was greatly stimulated by Schaudinn's³ discovery that the cause of syphilis was a spirochete.

Hoffmann and von Prowazek⁴ tentatively recognized three spirochetal forms in the mouth: (a) One relatively thick, 15 to $20\ \mu$ long, with a few smooth curves, the *Spirochæta buccalis*; (b) one extremely delicate, with very numerous (up to 20 or more), closely placed, sharp curves the *S. dentium*; and (c) a form intermediate between (a) and (b), a so-called "Mittelform." This last form in previous studies had apparently been confused with *S. dentium* and its separation represented a distinct advance. A year later von Prowazek⁵ applied the name *Spirochæta media* to this "Mittelform."

Three and possibly four oral spirochetes were recognized by Hartmann and Muehlens⁶ and by Muehlens⁷ viz: *Spirochæta buccalis*, *S. dentium*, *Spirochæta media*, in length, width, and nature of its curves occupying a position intermediate between *S. buccalis* and *S. dentium*, and *Spirochæta vincenti*. Muehlens was unable to convince himself that this last one was a species, distinct from *S. buccalis* or *S. media*. (See Fig. 78), I-M.

Gerber⁸ described six, not sharply defined, types of oral spirochetes. (Fig. 78, A, B, C, D, E, F.) For four of these he created new names. *Spirochæta inæqualis* (Gerber 1910), great irregularity in its curves, high and low, sharp and wide, usually 3 to 9 curves, very rarely 10 to 15 or more.

¹ Deutsch. med. Wchnschr., December 6, 1894, No. 49.

² Bull. et mém. Soc. méd. d. hôp., Paris, 1904, **21**, 1111; Lancet, 1905, i, 1260.

³ Deutsch. med. Wchnschr., 1905, **31**, 1728; Arb. a. d. k. Gsndhtsamte, 1905, **22**, 527.

⁴ Centralbl. f. Bakteriöl., Abt. I., Orig., 1906, **41**, 741, 817.

⁵ Arb. a. d. k. Gsndhtsamte, 1907, **26**, 26.

⁶ Ztschr. f. Hyg., 1906, **55**, 92.

⁷ Ztschr. f. Hyg., 1907, **57**, 412; Kolle und Wassermann's Handb. d. path. Microorganismen, 1913, **7**, 922, 927.

⁸ Centralbl. f. Bakteriöl., Abt. I., Orig., 1910, **56**, 508.

S. undulata (Gerber 1910), large coarse, regular curves, 2 to 7 in number, rarely more.

S. tenuis (Gerber 1910), so named for its extreme thinness or tenuity.

S. recta (Gerber 1910) even more tenuous than the preceding form, curves few and very low, so it forms almost a straight line, hence the name (*vd. Ozaki 1915, infra*).

The relationship of these morphologic types is tentatively set forth by Gerber as follows:

S. buccalis.

1. *S. undulata*.

2. *S. inæqualis*, possibly 1, in a condition analogous to rigor (Erstarrungsform).

S. dentium.

3. *S. dentium*.

4. *S. denticola*.

S. vincenti.

5. *S. tenuis*.

6. *S. recta*.

Gerber considers that forms 5 and 6 are possibly variants or degenerate forms of 1 and 2, or 1 or 2. Dobell¹ writes *à propos* of these views, "Gerber names no less than 6 species of spirochete from the human mouth and nasal cavities; but I find it absolutely impossible to identify these with my three forms." This seems rather an exaggeration, though there can be little doubt that the application by Gerber of scientific, specific names to morphologic types was uncalled for.

Bosanquet² published a little book which has been of some usefulness. He briefly describes (though any description at this time, 1911, would be inadequate) *Spirochæta buccalis* and *S. dentium*. He mentions *S. media* and *S. denticola*, and calls attention to a spirochete found in a case of abscess of the jaw, named by Veszpremi (1907) *Spirochæta gracilis*. This organism, however, he surmises, is identical with *S. vincenti*, which in turn so closely resembles *S. buccalis* that "some writers believe that they are identical" (p. 102).

A very sane review of the spirochetes of the mouth was issued in 1912 by Dobell.³ He recognized three species: (1) *Treponema buccale* (usually called *Spirochæta buccalis* Cohn); (2) *T. dentium* (usually *S. dentium* Koch) and (3) *T. intermedium*. He introduces

¹ *Vd. infra*, 1912, p. 161, f.n. 1.

² *Spirochætes*, Philadelphia, 1911.

³ *Arch. f. Protistenk.*, 1912, **26**, 119.

this last term for the "mittlere Spirochætenform" of Hoffmann and von Prowazek, apparently unaware that von Prowazek had already (1907) applied the name *Spirochæta media* to this form. Certainly the specific name, *intermedium*, is to be dropped on the rule of priority.

A morphologic classification of oral and dental spirochetes has been published by Erich Hoffmann.¹

1. *Spir. buccalis*, this includes the larger, coarser forms.
 - (a) *crassa*.
 - (b) *tenuis*.
 - (c) *inæqualis*.
2. *Spir. media oris* (E. Hoffmann u. von Prowazek). This includes the intermediate or "Mittelformen."
3. *Spir. dentium* (Koch) (*ev. orthodonta*).
4. *Spir. skoliiodonta*.
5. *Spir. trimerodonta* (= *Leptospira dentium*).

The three last forms (Nos. 3, 4 and 5) include the small fine, delicate spirochetes of the mouth. Unfortunately one is left in the dark respecting the peculiarities of the variety *crassa* and of the species *skoliiodonta*.

Kritchevski and Séguin² include among the spirochetes of Vincent's infection forms which they designate by the names *Spirochæta dentium*, *S. tenuis* and *S. acuta*. They are very imperfectly characterized. The first one agrees with the one described by Muehlens under the same name. The *S. tenuis* agrees with the *S. tenuis* of Gerber. The *S. acuta* shows resemblances to the *S. gracilis* Vez-premi, *Treponema macrodentium* and the form isolated by Ozaki (1915). It has tapering ends and a flattened spiral.

Present State of Our Knowledge of the Oral Spirochetes.—In 1918, Noguchi³ published a very fundamental analysis of the taxonomy of the spirochetes. His conclusions were accepted almost *in toto* by the Committee of the Society of American Bacteriologists which issued the *Manual of Determinative Bacteriology*, Baltimore, 1923. Six genera of spirochetes are recognized. The first three are not parasitic on man or other mammals and consequently may be left out of account here. The last three genera, *Spironema*, *Treponema* and *Leptospira* include all the species of interest to us from the standpoint of human pathology. In the second edition of the Manual (1925) the name *Borrelia* will supplant *Spironema*,

¹ Deutsch. med. Wchnschr., 1920, **46**, 257.

² Rev. de stomatol., 1920, **22**, 613.

³ Jour. Exper. Med., 1918, **27**, 575.

on account of priority. The characterizations of these genera given below are based on those in Bergey's Manual, 1923. (Fig. 29.)

Borrelia, a spiral flexible body with terminal filaments but no membrane.

Treponema, parasitic and frequently pathogenic forms with undulating or rigid spirilliform body. Without crista or columella. With or without flagelliform tapering ends.

Leptospira, parasitic forms, sharply twisted cylinders with flagelliform tapering ends, *one extremity being sharply curved into a "hook."*

At the present time it seems possible to admit the existence of at least five different spirochetal species resident in the mouth of man: *Borrelia buccalis* (Steinberg 1962), *Treponema microdentium* (Noguchi 1912), *T. macrodentium* (Noguchi 1912), *T. mucosum* (Noguchi 1912), and *Leptospira dentium* (Hoffmann, 1920). It is possible that two other species of the genus *Borrelia* may have to be recognized, *viz.*, *B. vincenti* and *B. bronchialis*.

Borrelia buccalis has been sufficiently characterized above in the chart. Personally I think the evidence is strong that the organism known commonly as Vincent's spirochete is a distinct species. As such it would be known as *Borrelia vincenti*. The assigning of this specific name is usually ascribed to Blanchard.¹ However as Blanchard gave no description this assignment seems unwarranted. There has long been uncertainty whether this organism constitutes an independent species or whether it is identical with or a variety of *Borrelia buccalis*. von Prowazek² simply states that the spirochetes associated with stomatitis are chiefly of the *buccalis* type. Muehlens³ held that it was impossible to decide whether Vincent's spirochete is an independent species or is to be identified with *Spirochæta buccalis* or *S. media*. Six years later he⁴ still thought it impossible to settle the question. Veszprémi⁵ thought that the spirochete of Vincent's angina is identical with the *Spirochæta buccalis*.

The first clear-cut statement that Vincent's spirochete is an independent species was made, as far as I can discover, by Thomson and Thomson⁶ (Fig. 78, N-R): "In our opinion *Spirochæta vincenti* (or what we consider to be *Spirochæta vincenti*) can be recognized as different from the *Spirochæta buccalis*, as it is a thinner spirochæte, not ribbon-like, and the coils are more irregular owing to the greater flexibility of the body."

¹ Arch. d. parasitol., 1906, **10**, 129.

² Arb. a. d. k. Gsndhtsamte, 1907, **26**, 26.

³ Zeitschr. f. Hyg., 1907, **57**, 413.

⁴ Kolle und Wasserman's Handb. d. path. Mikroorganismen, 1913, **7**, 927.

⁵ Quoted by Bosanquet: Loc. cit., 1911, p. 59.

⁶ Proc. Roy. Soc. Med., Marcus Beck Lab. Reports No. 3, VII, Pt. 1, p. 51.

Spirochetes.	Length.	Width.	Waves.				
			Char-acter.	No.	Regularity.	Flat.	Deep.
<i>Spirochæta buccalis</i> ¹	15-20 μ	Relatively thick	Smooth	Few	Fairly regular in living, resting state	..	
<i>Spirochæta buccalis</i> ²	12-20 μ	0.5-1 μ , Loeffler	Smooth	Few		..	
<i>Spirochæta buccalis</i> ³	10-20 μ	0.33-0.66 μ	Smooth	3-10	Uneven	..	
<i>Treponema buccale</i> , ⁴ S.	7-20 μ	Ca 0.3 μ	...	Very variable		..	
<i>buccalis</i> otherwise							
<i>Spirochæta buccalis</i> ⁵	10-20 μ	0.5-0.66 μ	...			..	
<i>Spirochæta buccalis</i> , ⁶ S.	10-20 μ , in dark-field	Ca. 0.1-0.2 μ ; is strikingly greater than width of other mouth spirochetes	Coarse, steep	Few (3-4 waves to every 10 μ)		..	
<i>buccalis</i> otherwise							
<i>Spirochæta vincenti</i> ⁷	Ca. 10 μ with 40 μ as extreme		...			..	
<i>Spirochæta vincenti</i> ⁸	8-20 μ		Smooth	3 or 4	Unequal; greater regularity when alive	..	
<i>Spirochæta dentium</i> ¹		Extremely delicate	...	Very numerous, up to 20 or more		..	Sharp
<i>Spirochæta dentium</i> ²	4-10 μ	Immeasurably thin up to 0.66 μ (Loeffler's)	...			..	
<i>Spirochæta dentium</i> ³	4-12 μ	0.25 μ at maximum	...	4 to 20	Regular	Flat	
<i>Treponema dentium</i> , ⁴ S.	4-18 μ , ca. 10 μ on average	Ca. 0.2 μ	...		Much more regular than T. buccale	..	
<i>dentium</i> otherwise							
<i>Spirochæta dentium</i> ⁵	4-12 μ		...	4 to 20	Moderately regular	Flat	
<i>Spirochæta dentium</i> , ⁶ S.	4-8 μ in dark-field	Probably less than 0.01-0.02 μ	...	4 to 5		..	
<i>dentium</i> otherwise							
<i>Treponema intermedium</i> ⁴	6-14 μ	Ca. 0.25 μ	...		More irregular than T. buccale	..	
<i>S. media</i> otherwise							
<i>Spirochæta media</i> , ⁶ S.	12-30 μ , or longer, in dark-field	Ca. 0.01-0.02 μ	Small	6 to 8 to every 10 μ		..	Shallow
<i>Spirochæta media</i> otherwise							

¹ von Prowazek and Hoffmann: Centralbl. f. Bakteriologie, 1906, 41, 741, 817.² Hartmann and Muehlens: Ztschr. f. Hyg., 1906, 55, 92.³ Muehlens: Ztschr. f. Hyg., 1907, 57, 412.⁴ Dobell: Arch. f. Protistenk., 1912, 26, 119.

Waves.				Ends.	Motility.	Miscellaneous.
Length.	Depth.	Tightly coiled.	Loosely coiled.			
Ca. 0.5 μ				Rounded or pointed		Terminal flagella and undulating membrane.
.....			Wide	Often pointed		Strongly refractile in life; blue to blue-violet by Giemsa; terminal flagellar processes and undulating membrane (Loeffler).
.....				Sharply pointed	Translation, rotation and flexion; often serpentine rather than screw-like	No flagella; circular in transverse apical section; no antero-posterior polarity; reproduction only by transverse division into two.
.....				Mostly rounded; are also slightly pointed	Active contraction of body and rotation	Thin periplastic processes (Loeffler's) and undulating membrane; very refractile when alive; blue to blue-violet (Giemsa).
.....				Pointed	Reversible corkscrew often accompanied by a progressive serpentine motion	
.....					Rather sluggish	
.....				Pointed	Active	
.....		Closely placed				
1.2 μ	0.33-0.66 μ	Angle of both limbs ca. 90 deg.				Very fine flagellar processes.
Short, 1.2 μ	0.33-0.66 μ			Often pointed		Flagella-like processes (Loeffler's); weakly refractile in life; red with Giemsa.
.....				Sharply pointed		No flagella; reproduce by transverse division, only.
.....				Often pointed		Flagella-like processes (Loeffler's).
.....				Tapering and pointed	Sometimes very active, of a coiling and twisting type, but more commonly only a slight corkscrew motion.	
.....			Looser than T. buccale	Usually more rounded than those of T. buccale		Reproduces by transverse division.
.....				Tapering and pointed	Less active than S. buccale; slow corkscrew rotation plus a gliding movement; no serpentine movement	

⁵ Muehlens in Kolle und Wassermann's Handb. d. path. Mikroorganismen, 1913, 7, 922.

⁶ Semple, Price-Jones and Digby: Jour. Roy. Army Med. Corps, 1919, 33, 287.

⁷ Bosanquet: Spirochaetes, Philadelphia, 1911.

Borrelia bronchialis is the name given to the spirochete found by Castellani in certain gangrenous and hemorrhagic, pulmonary infections. It morphologically closely resembles the Vincent's spirochete from which it has not as yet been sharply differentiated. Fantham¹ definitely asserts that *Borrelia bronchialis* is a species distinct from Vincent's or the other oral spirochetes.

Borrelia refringens Schaudinn 1905. This organism was first described from the genitalia of man. Spirals: 0.5 to 0.75 μ by 20 to 35 μ . The number of curves differs. The spiral amplitude is 3 μ . The ends are pointed with curved, flagella-like projections. The body of the organism is cylindrical. Strict anaërobe, cultivable in serum water containing fresh tissue. One often hears of "refringens type" spirochetes in the mouth, but this implies nothing more than morphologic similarity. Noguchi thought that this organism belonged to the genus *Treponema*, although easily differentiated from the species *pallidum*, *microdentium*, and *macrodentium*.

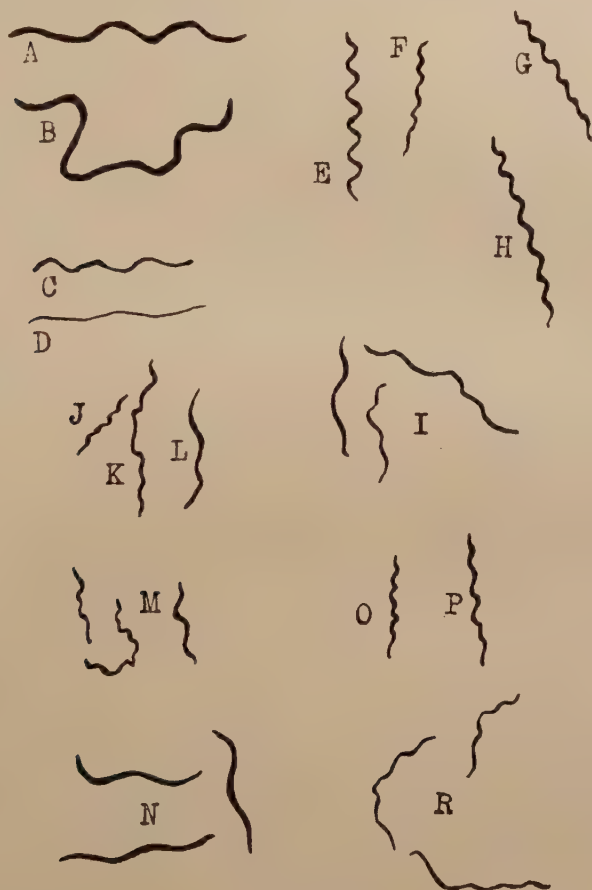
Treponema microdentium. (Fig. 78, G and O.) This organism was included in the old species *Spirochæta dentium* or *S. denticola* before Noguchi² isolated and carefully described it. Its diameter is less than 0.25 μ at its middle, tapering gradually off toward both extremities, which are sharply pointed: short in young cultures, reaching a length of 8 μ in old cultures. On the average 14 curves. The pointed ends are usually drawn out straight along the hypothetical axis. Active, rotary motility. Body flexible. Along this flagellum-like projection is at times observed at one or both ends. Longitudinal division noted. There is a loose coagulation of the serum constituents of the medium, and litmus becomes colorless after about two weeks growth. Cultures exhale a fetid odor.

Treponema macrodentium. (Fig. 78, H and P.)—This is probably the *Spirochæta media* of von Prowazek (1907). In serum-agar-tissue medium it appears after about four or five days at 37° C. as a faint, hazy colony, almost transparent and without demarcation. In young cultures the organisms are plump and short with rather irregular shallow curves. The extremities taper off abruptly. Motility, swinging or vigorously vibrating, long delicate flagellum-like projections, with minute curves, attached to one or both ends. Under dark-field illumination the body shows double refraction along the axis, bordered on both sides with stronger zones of refraction. Two to 8 curves. Width, 0.7 to 1.0 μ ; length 3 to 8 μ .

¹ Ann. Trop. Med. and Parasitol., 1915, 9, 402.

² Jour. Exper. Med., 1912, 15, 81.

In older cultures, the organisms are somewhat longer and thinner and taper more gradually toward the ends; the curves are almost



ORAL SPIROCHETES.

FIG. 78.—A–F. After Gerber (1910). $\times$ about 1700. A, *Spirochaeta undulata*; B, *S. inaequalis*; C, *S. tenuis*; D, *S. recta*; E, *S. dentium*; F, *S. denticola*.

G–H. After Noguchi (1912). $\times$ about 2100. G, *Treponema microdentium*; H, *T. macrodentium*.

I–M. After Muehlens (1907). $\times$ about 1350. I, Vincent's spirochetes; J, *Spirochaeta dentium*; K, *S. buccalis*; L, *S. media* ("mittlere Form"); M, *S. dentium* from pure culture.

N–R. After Thompson and Thompson (1914). $\times$ about 1500. N, *Spirochaeta buccalis*; O, *Treponema microdentium*; P, *T. macrodentium*; R, *Spirochaeta vincenti*.

rectangular, shallow, and quite regular. No double refraction except near middle. Fourteen or more curves. Width, $0.3\ \mu$; length, $12\ \mu$ or more. Longitudinal division noted.

Treponema mucosum.—This organism was also isolated and carefully described by Noguchi.¹ Up until then it probably had been included in the species known as *Spirochæta dentium*. Morphologically resembles both *T. pallidum* and *T. microdentium*; 8 to 12 μ by 0.25 to 0.3 μ . Number of curves varies from 6 to 8. These curves are remarkably regular and are often quite deep. Both extremities are sharply pointed and often possess a fine, minutely curved projection that varies in length in different organisms. The length of this projection may reach 8 to 10 μ . In its staining reactions. *T. mucosum* behaves as does *T. microdentium*, taking the red in the Giemsa stain. Differentiation: *T. mucosum* differs from *T. microdentium* in producing a mucin, has a stronger fetid odor, a denser growth, and in surviving in the rabbit testicle when introduced with agar. *T. pallidum* differs from *T. mucosum* by its pathogenicity, by producing no odor or mucin, by its fainter diffuse growth, and by requiring for its growth the presence of fresh tissue in the media.

Leptospira dentium.—Attention was first called to this organism by Erich Hoffmann² under the name of *Spirochæta trimerodonta*. A little later he³ changed the name to *Leptospira dentium*, conformably with Noguchi's classification.⁴ Edmund Hofmann reported that this organism was found in the mouths of about 40 per cent of an unselected series. Its numbers however seem to be much decreased or it may even be absent when the bacterial or spirochetel flora (or fauna) of the mouth is unusually abundant as *e. g.* in Vincent's stomatitis. He found this species resistant to saponin. Representatives of the genus *Leptospira* have likewise been noted by Perrin⁵ who found them in 35 per cent of cases of "pyorrhea alveolaris."

Other Oral Spirochetes.—A number of reports have appeared describing the isolation of spirochetes from the human mouth. Unfortunately I am unable to identify them with any of the forms described above. Repaci⁶ isolated by Veillon's technic a strict anaërobe. On the original culture it appears toward the eighth day after inoculation. Its colonies are small, translucent, dew-drop points. Its optimum temperature is 37° C. No gas is formed in the media. Bouillon remains clear. It exerts no recognizable effect

¹ Jour. Exper. Med., 1912, **16**, 194.

² Deutsch. med. Wchnschr., 1920, **46**, 257.

³ Ibid., 1920, **46**, 525.

⁴ Jour. Exper. Med., 1918, **27**, 575.

⁵ Rev. mex. de biol., 1922, **2**, 171.

⁶ Compt. rend. Soc. de biol., 1911, **70**, 784.

on glucose, saccharose or dextrin. Lactose is slowly acidified but no coagulation occurs. Its curves are preformed, *i. e.*, they do not disappear when the organism is at rest. Their number is very variable; they are regular and parallel. Their depth is about $1\ \mu$ and their amplitude 1 to $2\ \mu$. The thickness of the organism is from 0.66 to $1.0\ \mu$. It is easily stained by the ordinary dyes, is Gram-negative. With Giemsa it takes a bluish tint and cilia are demonstrable by Loeffler's method. Rapaci considers that this organism is clearly different from *Spirochæta buccalis* or *S. dentium*. *S. buccalis* is thicker and its curves are fewer and looser. In Repaci's opinion this organism is intermediate between *S. buccalis* and *S. dentium*. However by this it is no means clear that he intends to suggest that his organism is, or is related to, the *S. media* of von Prowazek. Dobell,¹ however, identifies Repaci's spirochete as this *S. media*.

Another spirochete whose isolation was reported by Ozaki² is also an anaërobe. It may be isolated on half-coagulated horse-serum. It is strictly mesophilic. The colonies are very hazy and cloudy. It does not liquefy the serum medium. No gas is formed. On ascites-agar (1 to 1) on the third day after inoculation growth appears as an obvious, cloudy region which rapidly enlarges and becomes somewhat opaque. Neither indol nor H_2S is formed. No odor is recognizable in the media until after two or three weeks when a very slight, disagreeable one becomes apparent. It is very polymorphic from soft ascites-agar (1 to 1) and half-coagulated horse serum. From horse serum, it is 4 to $8\ \mu$ long. Its thickness, when stained by Giemsa, is 0.33 to $0.5\ \mu$. Its curves are very uneven; their number and amplitude are very irregular, usually only 3 or 4, though in longer specimens the number of curves may reach 8. Examined by dark-field illumination no definite locomotion is demonstrable though twistings and sudden, convulsive movements are clearly seen. Ozaki's spirochete among other things is distinguished by the remarkable flatness of its curves. This suggests the *Spirochæta recta* of Gerber (1910). Ozaki is sure that his spirochete is not closely related to Repaci's, but does not commit himself upon the question whether the spirochete isolated by him is to be identified with any of the previously described oral species or is a new one.

Cavalié and Mandoul³ announced as a new species, a "Spirochæte

¹ Arch. f. Protistenk., 1912, **26**, 119.

² Centralbl. f. Bakteriöl., Abt. I., Orig., 1915, **76**, 469.

³ Compt. rend. Soc. de biol., 1921, **85**, 1068.

perforans." They had observed it constantly in the lesions of "pyorrhea alveolaris." Length 10 to 13 μ . Thickness about 2 μ . Ends not tapering. No flagella apparent. In some specimens, with 3 to 5 curves, the amplitude is great and the height of the curves is low. In other specimens as many as 9 curves are present. From the meagreness of the description and because its differences from other forms are not particularized, I do not think we are justified in accepting it as proven that it is here a question of a new species.

Pons¹ isolated in horse serum diluted with Ringer's solution (1 to 9) a microorganism which he groups with the spirochetes. If this be warranted it holds a unique position in that after isolation it can be grown on the ordinary culture media. Its length is from 2 to 10 to 15 μ ; its width 0.2 to 0.5 μ . It is very motile, without rotation around its long axis. It is Gram-negative and stains easily with the ordinary aniline dyes. With Giemsa it assumes a blue violet tint. The majority of individuals from a forty-eight-hour culture have 1 or 2 curves; in older cultures the number increases up to 5. At forty-eight hours the cultures emit a very putrid odor. Bouillon is cloudy in forty-eight hours, no surface growth but a whitish ring adherent to the tube, with the consistency of mucus. In time an abundant, grayish, mucoid deposit collects. On agar at twenty-four hours the colonies are small, transparent, granular; later becoming opaque, circular, and slightly yellow. No liquefaction in gelatin coagulated serum. No indol. Ferments no carbohydrate. Very slightly proteolytic. Optimum temperature 37° C. Reproduction is by transverse division and by granules.

Cultivation and Isolation of Oral Spirochetes.—This has been done by Weaver and Tunnicliff,² Tunnicliff,³ Muehlens,⁴ Muehlens and Hartmann,⁵ Repaci,⁶ Noguchi,⁷ Shmamine,⁸ Ozaki⁹ and by Pons.¹⁰

Pathogenicity of Oral Spirochetes.—There is no convincing evidence that these forms are capable of producing disease in man. This statement, however, is not to be considered as equivalent to saying that they are non-pathogenic. Muehlens and Hartmann¹¹ found

¹ Compt. rend. Soc. de biol., 1924, **91**, 150.

² Jour. Infect. Dis., 1905, **2**, 446.

³ Ibid., 1906, **3**, 148.

⁴ Deutsch. med. Wchnschr., 1906, No. 20.

⁵ Ztschr. f. Hyg., 1906, **55**, 81.

⁶ Compt. rend. Soc. de biol., 1911, **70**, 784.

⁷ Jour. Exper. Med., 1912, **15**, 81; *ibid.*, 1912, **16**, 194.

⁸ Centralbl. f. Bakteriolog., Abt. I., Orig., 1912, **65**, 311.

⁹ *Ibid.*, 1915, **76**, 469.

¹⁰ Compt. rend. Soc. de biol., 1924, **91**, 150.

¹¹ Zeitschr. f. Hyg., 1906, **55**, 81.

their cultures of *Spirochæta dentium* of Hoffmann and von Prowazek non-pathogenic when injected intraperitoneally, subcutaneously, intramuscularly, intravenously, intracorneally and intra-ocularly into rabbits, guinea-pigs and mice. *Treponema microdentium*¹ injected into the testicle of the rabbit or subcutaneously into the monkey, *Macacus rhesus*, caused within twenty-four hours a marked induration persisting for about one week; but no spirochetes were detected in the inflamed tissue. *Treponema mucosum*² can call forth only a local and transient reaction in *Macacus rhesus*. It can survive in the testicle of the rabbit when injected simultaneously with some foreign substance, *e. g.*, agar.

The organism, isolated and identified by Shmamine³ as *Spirochæta dentium* proved to be non-pathogenic for such laboratory animals on which it was tested. The kinds of animals are not specified in the original.

Ozaki's⁴ organism was almost non-pathogenic ("fast nicht pathogen") for rabbits, guinea-pigs and mice.

The organism isolated by Pons⁵ rapidly produced on subcutaneous inoculation into guinea-pigs a gelatinous edema, and later a septicemia. Blood cultures were positive. It also was capable of producing a peritonitis with false membranes and death in three to five days.

Clinical opinion in general is in favor of regarding Vincent's spirochete as pathogenic for man when conditions are suitable. Although its invariable presence in certain types of lesions, its vast increase in numbers in these lesions, its position in the lesions as shown in histological examination, and its disappearance *pari passu* with the healing of the lesions—all speak for its pathogenicity, this has not been established beyond all doubt.

¹ Noguchi: Jour. Exper. Med., 1912, **15**, 81.

² Noguchi: Ibid., **16**, 194.

³ Centralbl. f. Bakteriöl., Abt. I., Orig., 1912, **65**, 311.

⁴ Ibid., 1915, **76**, 469.

⁵ Compt. rend. Soc. de biol., 1924, **91**, 150.

CHAPTER XX.

DENTAL CARIES.

DENTAL caries is a complex process. Many factors play a part in its production. Some of these factors are known, some surmised. Some are predisposing, some exciting; some general, some local. The complete story of the causation of dental caries cannot yet be written. In the light of present knowledge, and not in the least contradicted or superseded by the more recently developed dietary considerations, one *indispensable* factor is the activity of certain bacteria. Miller¹ first clearly defined the role of these microorganisms in this connection and the author is aware of no cogent evidence to justify any essential modification of Miller's chemico-parasitic theory. This theory, it is recognized, does not by any means cover the whole problem. We are still uncertain why dental caries begins as a localized disintegration instead of attacking the entire enamel surface, and the whole question of immunity and susceptibility lies unanswered. But insofar as they go, *i. e.*, by pointing out the part played by bacteria, Miller's views still appear valid and they have proved to be useful.

The characteristics of a pathological process are in part determined by the architecture and chemistry of the organ or tissue in which it runs its course. Even the little we know about dental caries cannot be understood without reference to the structure and composition of the enamel and dentin. The inorganic content² of the enamel is so high that when the lime salts have been dissolved, practically nothing is left. In the dentin, the organic content is high enough so that even after the lime salts have been dissolved, the form of the dentin body is still preserved intact. Consequently in the case of the dentin not only must inorganic constituents be removed but the organic matrix must be in some way destroyed, before we can find a cavity in the tooth. It might also be surmised that the agent which dissolves the inorganic

¹ Summarized in *Die Mikro-organismen der Mundhöhle*, 2d ed., Leipzig, 1892.

² Evans (XVII Internat. Congr. Med., London, Sect. Stomat., 1913, 17, 73) on the basis of analyses of two samples of human enamel (age of patient or time after extraction not given) estimated that the amount of organic matter is from 1 to 2 per cent at least.

salts is not the agent which dissolves the organic matrix of the dentin.

The inorganic salts of the enamel and dentin are dissolved by acids, in whose production bacteria are indirectly concerned. The bacteria produce enzymes (amylolytic and sugar-splitting) which enormously accelerate the rate at which carbohydrates are broken down. Some of the products of this disintegration are acids. The source of these carbohydrates according to the view most widely held at present is the food débris of the mouth, although this problem has not yet been settled satisfactorily. The acids concerned are organic, of which lactic is usually regarded as predominant. Sperling¹ found in this connection pure dextrorotary lactic acid. Dodds,² however, found malic acid to be the chief acid product of *B. acidophilus odontolyticus*. Langwill³ found that the non-volatile acid produced by hemolytic and non-hemolytic streptococci was almost entirely lactic acid of the racemic type. The non-hemolytic streptococci produced formic, acetic and a possible trace of butyric acid. The hemolytic and *viridans* types produced acetic and propionic acids. Butyric is probably present in small quantities; and all tests for formic acid gave negative results.

The ability to form enzymes which mediate in the disintegration of carbohydrates with the resulting formation of organic acids, is a property widely distributed even among very different species of bacteria. Such bacteria are common in soil, water, foods and air, so their presence in the mouth is not a mystery. When capable of thriving in the environment of the mouth, any or all of these species may contribute to the decalcification of the hard dental tissues.

The decalcification of the enamel completes the destruction of this tissue. The decalcification of the dentin must be supplemented by another process. The organic matrix of the dentin, that part remaining after the inorganic salts have been dissolved out, can be digested and liquefied by the direct action of the proteolytic or proteocalstic enzymes of bacteria. This property of certain bacteria is exemplified by the liquefaction of nutrient gelatin culture medium or of the casein clot of milk. Although one should hesitate to infer from the liquefaction of gelatin or casein that a particular microörganism can likewise digest the

¹ Deutsch. Monatschrft. f. Zahnk., 1922, 40, 129.

² Brit. Jour. Exper. Path., 1924, 5, 183.

³ Jour. Bacteriol., 1924, 9, 79.



FIG. 79.—F, interglobular spaces in dentin beneath enamel. The general trend of the dentinal tubuli toward the pulp is also shown. (Hopewell-Smith.)

organic matrix of dentin, nevertheless this organic matrix is digested by bacterial proteolytic enzymes elaborated by a wide range of species. The products of this digestion are dissolved in and washed away by the saliva. The result is the cavity in the tooth. Although the decalcification and the proteolysis of the organic matrix are such different processes that it usually has been assumed that two different bacterial groups were respectively necessary, it may well be that one and the same species can perform both functions. For example, the ordinary pyogenic staphylococci form acids from carbohydrates and elaborate proteolytic enzymes; and more pertinently McIntosh, James and Lazarus-Barlow (see *infra*) describe the decalcification and liquefaction of dentin experimentally by pure cultures of aciduric bacilli.

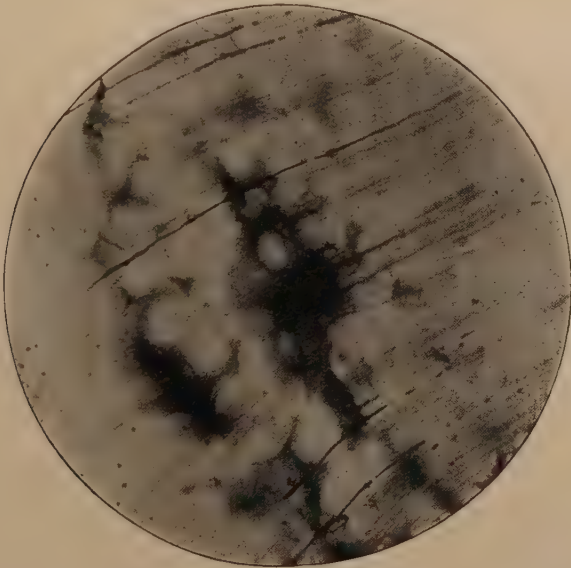


Fig. 80.—Interglobular spaces in dentin. $\times 240$. (Hopewell-Smith.)

The structure of the dentin determines in part the course of the bacterial invasion. The interglobular spaces of Czermak in the dentin (Figs. 79 and 80) just under the enamel constitute a line of weakness. The bacteria reaching this zone spread out and undermine the enamel. The result of this process is frequently seen in the clinic where an extensive cavity is found with an astonishing small orifice communicating with the mouth. The second histological peculiarity is the tubular structure. The lumen of the dentinal

tubule has a diameter amply sufficient for the passage of most bacterial cells. This fact together with the arrangement of the tubules approximately at right angles to the surface of the pulp makes it very easy for the bacteria to progress toward the pulp (Fig. 81). The result is that pulpal infection always occurs before actual, gross pulp exposure. In any tooth in which dental caries has involved the dentin, the pulp must be regarded as at least potentially infected. The fact that the bacteria have always penetrated farther into the dentinal tubuli than is indicated by any gross change in the dentin necessitates in cavity preparation that attention be given to sterilizing the cavity wall before the

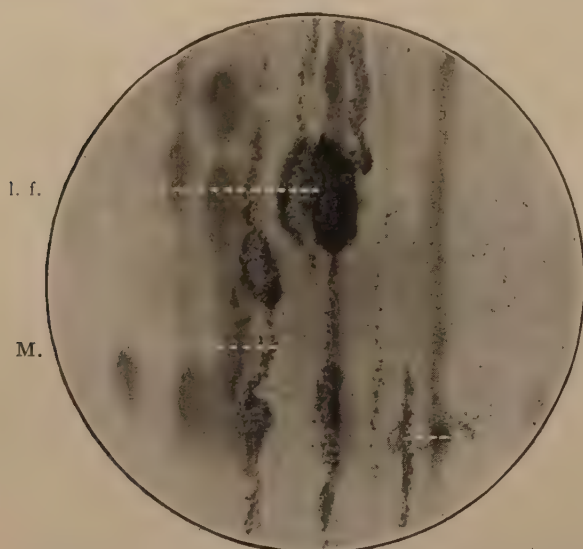


FIG. 81.—Bacteria in the dentinal tubuli (M) and "liquefaction focus" l.f.). $\times 450$.
(Hopewell-Smith.)

filling material be introduced. The discoloration of carious dentin is an incidental by-process and is ascribed to certain chromogenic saprophytes; though the absorption of coloring matters from food and drink and blood would seem to offer an equally plausible explanation.

THE BACTERIAL PLACQUE.

Reference has already been made to the fact that dental caries begins as a process limited to a small area on the free surface of a tooth. This localization cannot apparently always be accounted

for by the presence of developmental defects. To furnish a plausible mechanism for some instances of this localization the concept of the bacterial plaque was introduced, and there is some histological evidence for its existence. The simplest and most satisfactory suggestion regarding the method of formation of such plaques was advanced by Kirk. A clump of bacteria temporarily lodged on the enamel through their enzymes act on carbohydrates, liberating organic acids. These acids precipitate the mucin from the saliva, which carries with it particles of food débris, gluing the bacteria to that particular spot. The bacteria, mucin and food particles together constitute the plaque. The process is continued, resulting in a larger plaque, which protects the multiplying bacteria, localizes their activities and to some extent by interfering with the rapid diffusion into the saliva permits a concentration of the acids.

THE BACTERIA OF DENTAL CARIES.

The available evidence favors the view that dental caries is not a *specific* bacterial disease. Typhoid fever is a specific bacterial disease because the typhoid bacillus is absolutely necessary for its production; dental caries is *not* a specific bacterial disease because the indispensable part played by bacteria may apparently be met by any one or several or a rather large group of species. It is probable that microorganisms which rapidly split up carbohydrates with the liberation of relatively large quantities of organic acids and which can tolerate high acid concentrations are the more effective and important in the process of decalcification. It is also probable that different bacteria digest the organic matrix of the decalcified dentin at different rates and in this case the possession of marked proteoclastic power would enhance the importance of its possessor in this phase of dental caries. The significance of these considerations will become apparent in the following enumeration and description of the various kinds of bacteria which have been isolated from carious teeth.

Before considering particular species, some general remarks on the oral flora in health and in cases of dental caries are in order. Kliger's studies¹ show that the average number of bacteria per milligram of material examined microscopically was 10,500,000 in cases immune to dental caries. In cases where the enamel alone was affected the number was 530,000,000 and where enamel and dentin

¹ Jour. Allied Dent. Soc., 1915, **10**, 141, 282 and 445 (Table X, p. 302).

both were affected the number was 258,900,000. These figures show clearly that the carious process is associated with a number of bacteria enormously exceeding that on the surfaces of teeth in caries-immune mouths:

Description of group.	Microscope count, average number per mg.
Immune cases	10,500,000
Healthy teeth, "clean" mouths	24,500,000
Healthy teeth, "dirty" mouths	47,000,000
Healthy teeth, "dirty" mouths, tobacco chewers	25,000,000
Healthy teeth, before brushing	68,000,000
Healthy teeth, after brushing	16,250,000
Healthy teeth, before meal	26,500,000
Healthy teeth, after meal	67,500,000
Primary caries	530,000,000
Caries, enamel and dentin	258,900,000
Pulp exposed, superficial layer	37,200,000
Pulp putrescent, deep (layer, third and fourth stage of decay)	57,670,000

Besides these quantitative changes, Kligler also noted changes in the types of bacteria. In deposits on normal teeth the cocci were most prevalent, constituting about 75 per cent of the total flora. Forty per cent of the cocci were streptococci. In the primary stage of dental caries, only 40 to 50 per cent of the forms were cocci while the percentage of thread forms rose to about 30 per cent (an increase of about 200 per cent), accompanied by a correspondingly large increase in the number of asporogenic, actively acid-producing rods.

Goadby (see *infra* p. 138) notes that in making cultivations from the mouths of natives (presumably caries-immune Zulus and Kaffirs) and also from the mouths of some monkeys, he was struck with the number of putrefactive rather than acid-forming bacteria present. On the other hand, Pickerill and Champaloup¹ found evidence "that there exists no great difference between the oral flora of immune and of susceptible individuals, and that *freedom from caries in such children is not due to the absence of those organisms which are usually regarded as causal factors.*"

Although Miller's views on the part played by bacteria in the decay of teeth are all but universally accepted today, one cannot recognize with any feeling of certitude the identity of the various microorganisms mentioned as of possible etiological importance. This condition is ascribable to the state of bacteriological technic of his time. We lose little but what is only of historical interest, if we begin our enumeration of particular bacterial species with a consideration of Goadby's work. This was summarized in his

¹ Pickerill: *Prevention of Dental Caries*, 3d ed., 1924, p. 31.

Mycology of the Mouth, published in 1903. His findings are given in the following table.

BACTERIA OF DENTAL CARIES.

ACID-FORMING BACTERIA.

Streptococcus	}	Deep layers of carious dentin.
B. necrodentalis		
Staphylococcus albus		
Streptococcus brevis		
Sarcina lutea	}	Superficial layers of carious dentin.
Sarcina aurantiaca		
Sarcina alba (Eisenberg)		
Staphylococcus albus		
Staphylococcus aureus		

BACTERIA WHICH LIQUEFY DENTIN (DECALCIFIED).

Non isolated as yet	}	Deep layers of carious dentin.
B. mesentericus ruber		
B. mesentericus vulgatus	}	Superficial layers of carious dentin.
B. mesentericus fuscus		
B. furvus		
B. gingivæ pyogenes		
B. liquefasciens fluorescens motilis		
B. subtilis		
Proteus Zenkeri		
B. plexiformis		

Just after Goadby's publication, Rodella¹ reported an anaërobic *B. putrificus* (Bienstock) in carious dentin. This author ascribed to this organism and to the anaërobic butyric acid bacilli the important part in dental caries.

Goadby and Barrett² report the isolation of the following micro-organisms with their corresponding percentages from cavities both shallow and deep, but not extending to the pulp. The surface layer of dentin had been removed with the idea of minimizing accidental contamination. Only freshly extracted teeth, the subjects of acute pain at the time of removal (acute pulpitis without gangrene), were used. The surrounding tissues of these teeth showed no inflammation. Under these conditions the following organisms were found: Micrococci (90 per cent), streptococci (66 per cent), staphylococci (40 per cent), the *necrodentalis* group (80 per cent), streptobacilli (50 per cent), threads (90 per cent) and yeasts (33 per cent). In no case were pure cultures of the thread forms obtained, and those which were isolated were strict anaërobes.

In the hard but discolored dentin at the base of the cavity, the following organisms were encountered: Micrococci (20 per cent),

¹ Arch. f. Hyg., 1905, **53**, 329.

² XVII Internat. Congr. Med., London, Sect. Stomat., 1913, **17**, 31.

Streptococcus brevis (20 per cent), *Streptococcus conglomeratus* (40 per cent), the *necrodentalis* group (80 per cent), *B. mesentericus* (20 per cent), streptobacilli (80 per cent) and yeasts (60 per cent). Goadby and Barrett note as two points of special interest in this list: (1) The presence of the *mesentericus* group in the deep layers of carious dentin, and (2) the large number of streptobacilli found. These latter organisms were once confused with streptococci, but they are regarded as being related to the *necrodentalis* group.

Dellevie¹ found streptococci with considerable frequency in the deep layers of dentin. Sieberth² made essentially the same observation in an examination of 16 teeth. Baumgartner³ considered the streptococcus to be the organism of prime importance in the initiation of the disintegration of the enamel. Kantorowicz⁴ observed that the flora of dental caries becomes the more uniform as the deeper layers are investigated. Three groups were found at the deepest levels: (1) Staphylococci which did not liquefy gelatin; (2) streptococci, and (3) straight rods, one of which was perhaps identical with Goadby's *B. necrodentalis*. In relative numbers the streptococci far exceed all others; the rods occupy second place in frequency and the staphylococci last. Bacteria, having the property of liquefying gelatin, were never cultivated from the deepest, but only from the middle and more superficial layers. Kantorowicz distinguishes two kinds of streptococci which he designates as *Streptococcus a* and *b*. Hilgers⁵ decides that this distinction of an *a* and a *b* type is unnecessary, as both types interblend. Becker⁶ regards *a* as a mutation of *b* but denies that *b* can be derived from *a*. He supports the validity of Kantorowicz's differentiation. The rod-shaped bacteria of Kantorowicz belong to the group of the so-called lactic acid formers.

The easily identifiable species found in the superficial layers of carious dentin, are according to this author: *Staphylococcus albus* (very frequently), *S. citreus* (more rarely), *S. aureus* (only once), yeasts (frequently), *sarcinae* (occasionally), *B. subtilis* and *B. mesentericus* (both occasionally) and *B. proteus* (frequently). Kantorowicz regards the streptococci as the chief bacterial agents

¹ Ueber die Bedeutung der Antisepsis in Munde, Inaug. Diss., Berlin, 1891.

² Die Mikro-organismen der Zahnpulpa, Inaug. Diss., Erlangen, 1900.

³ Ueber das Wesen der Zahnkaries, mit besonderer Beruecksichtigung der Histologie des gesunden und cariösen Zahnschmelzes, Deutsch. Monatschr. f. Zahnheilk., 1911, H. 5; Wien. klin. Wchnschr., 1913, 26, 178.

⁴ Bakteriologische und histologische Studien ueber die Caries des Dentins, Deutsch. Zahnheilk., 1911, H. 21.

⁵ Deutsch. Monatschr. f. Zahnheilk., 1921, 39, 357.

⁶ Ibid., 1923, 41, 308.

in the production of dental caries. His views, judging from his *Klinische Zahnheilkunde* (Berlin, 1924, 421), remain essentially unchanged. He makes no reference to the work of Klinger, Howe and Hatch, and McIntosh, James and Lazarus-Barlow.

The closeness of the results of the studies of Goadby and of those of Kantorowicz deserve attention. If we ignore the specific terms "brevis" and "albus" of Goadby, his group of the acid-forming bacteria from the deep layers of carious dentin differs very little from the group isolated from the same levels by the German investigator. Goadby's streptococcus, *B. necrodentalis*, and staphylococcus correspond well with Kantorowicz's streptococci, "lactic acid" bacilli, and non-liquefying staphylococci. Further, both observers noted that liquefying bacteria had not been recovered from the deep layers of carious dentin.

Niedergesaess¹ selected teeth for the study of the bacteriology of dental caries which had only small cavities and in which no kind of pulpal disease was present. On direct microscopical examination of the most superficial carious layers a rich and varied bacterial flora was found. In the vast majority of cases rods predominated (short, long, delicate and thick), associated with a few staphylococci, very rare streptococci and an occasional sarcina or spirillum. In the deeper carious layers there were more micrococci than rods. The deeper one went the cocci increased and the rods gradually disappeared. In the very deepest layers only cocci were seen. On cultural examination it appeared that streptococci were always present. These usually proved to be slightly pathogenic, long-chained, mostly strongly hemolytic and bile-insoluble. Twice *Staphylococcus aureus* was recovered from these deepest levels and twice streptococcal strains which were regarded as *Streptococcus acidilactici* on account of their oval outline, short chains in glucose bouillon, weak or absent hemolysis and partial bile-solubility. Hilgers² assumes the streptococcal nature of dental caries and in his studies of the streptococci isolated from carious material believes he recognizes two groups: (1) a *pyogenes* group conspicuous in cultures made soon after extraction and (2) a *lacticus* group which appears in increasing numbers, the longer the tooth has been out of the mouth. Twenty days after extraction the *lacticus* forms alone are cultivable. The observations, made apparently independently, closely agree with the findings of Niedergesaess. The

¹ Anatomische, bakteriologische und chemische Untersuchungen ueber die Entstehung der Zahnkaries, Arch. f. Hyg., 1915, **84**, 221.

² Deutsch. Monatschrft. f. Zahnk., 1915, **39**, 357.

predominant streptococcus of this author would belong to the *pyogenes* group and the two strains identified as *S. acidilactici* would belong to the *lacticus* group of Hilgers. In a later study¹ Hilgers has isolated also Gram-positive, non-motile, asporogenic, acidific and acidophilic rods which he identified as the *B. necrodentalis* of Goadby.

Sperling² concluded that the *Streptococcus lacticus* (Kruse) is the only important microorganism in the production of dental caries. The Gram-positive rods which he also found in the deepest layers of carious dentin he regards as variations of this streptococcus because of apparent transmutations occurring in presumably pure cultures.

Clarke,³ in carefully made studies isolated *B. acidophilus* and what he regarded as a previously undescribed streptococcus with some frequency from carious material. Ordinary mouth streptococci, mainly of the *S. salivarius* type, were encountered, and also but with considerable rarity, staphylococci. The peculiar streptococcus he named *S. mutans* and regarded it as the bacterial factor in dental caries. It was found more frequently than *B. acidophilus*, although it was not isolated in all instances (present in cultures from 36 of 50 teeth). Its colonies have the property of adhering closely to the surfaces of teeth, a property stressed by Kliger for his *Cladothrix placoides* and *Leptothrices* (see *infra*). Clarke believes that it is experimentally demonstrable that *S. mutans* is able to decalcify enamel and penetrate dentin under conditions approximating those in the mouth, which in his opinion cannot be said for *B. acidophilus*.

Ludwig Heim⁴ reported on some studies on the bacteriology of dental caries. Eighteen carious teeth were examined. Streptococci, for the most part in short-chains, were found 15 times, and Gram-positive, asporogenic rods were found 13 times. These rods apparently were close relatives of *B. necrodentalis* or *B. acidophilus*. A short note⁵ upon the continuation of this study contains essentially the same information.

The studies published by Kliger⁶ are unsurpassed for their painstaking care and comprehensiveness. Reference has already been made to the fact that the bacterial content of deposits on the teeth

¹ Centralbl. f. Bakteriöl., I. Abt., Orig., 1924, **93**, 249.

² Der Streptococcus lacticus (Kruse) in seiner Beziehung zur Zahnkaries, Maerz, Deutsch. Monatschr. f. Zahnk., 1922, **40**, 129.

³ On the Bacterial Factor in the Etiology of Dental Caries, Brit. Jour. Exper. Path., 1924, **5**, 141.

⁴ Sitzungsber. d. physik.-med. Soz. in Erlangen, 1922-1923, **54-55**, 121.

⁵ Centralbl. f. Bakteriöl., I. Abt., Orig., 1924, **93**, 252.

⁶ Jour. Allied Dent. Soc., 1915, **10**, 141, 282 and 445.

is enormously increased in dental caries, *e. g.*, from the 10,500,000 organisms per milligram found in caries-immune mouths to over 500,000,000 in primary caries or to over a 250,000,000 in dental caries involving the dentin. Beside this quantitative change, there was a decided alteration in the relative abundance of types. The cocci were most abundant on normal teeth, representing about 75 per cent of the total flora. Forty per cent of the cocci were streptococci. On the other hand, in the primary stage of dental caries (enamel alone affected) only 40 to 50 per cent were cocci while the percentage of thread forms rose to about 30 per cent (an increase of about 200 per cent), accompanied by a correspondingly large increase in the number of the asporogenic, actively acid-producing rods. This decrease in the cocci and especially in the streptococci does not tend to support the views of those who assign to this latter group the chief part in the decalcificatory phase of dental caries. Kliger specifically names (p. 457) the three forms prominent in carious enamel deposits: *Bacillus acidophilus*, *Cladothrix placoides* and *Leptothrix buccalis*. A peculiar significance lies in these species. The first belongs to a group that has exceptional powers of forming acids from carbohydrates, *e. g.*, producing and tolerating an acidity of 8 per cent normal acid. The two latter species, while also having acid-forming ability, are distinguished by the readiness with which they can attach themselves in the form of compact colonies to any surface.

Kliger remarked (p. 313) that the *B. necrodentalis* of Goadby closely resembled the peculiarly acidific strains encountered in his own work. This view is tentatively accepted by Hadley.¹ The identity of the *B. necrodentalis* of dental caries with the *B. acidophilus* of the intestine has been serologically (agglutinins and antihemolysins) proved by Hilgers.² Howe and Hatch³ directed much attention to this group. Their approach to the problem was novel. Chemically inert fillings were inserted over the carious mass in the tooth. "It seemed probable that the extraneous flora would die off, while the flora that had the best hold upon life in this environment would survive." The study was conducted also under other conditions but this seems the most pertinent. Six weeks to three months later the fillings were removed and the underlying carious dentin cultured. Where the three-month interval had lapsed, no other microorganisms than those of the markedly acidific Moro-Tissier

¹ Bacteriology of Dental Caries, Dent. Cosmos, 1924, **66**, 715.

² Centralbl. f. Bakteriöl., I. Abt., Orig., 1924, **93**, 249.

³ Study of the Microorganisms of Dental Caries, Jour. Med. Res., 1917, **31**, 481; reprinted in the Dent. Cosmos, 1917, **59**, 961.

group were found. To Howe and Hatch this constitutes the constant and predominant flora of dental caries. The organisms isolated were identified or named as follows: *Lactobacillus acidophilus* (Moro), *Bacillus X*, *Bacillus M*, *Bacillus Y* and *Bacteroides bifidus*.



FIG. 82.—*B. bifidus*, representing the various forms described; the irregularly stained or vesicular forms being from old cultures. $\times$ about 1800 diameters. (Park and Williams.)

The probability that certain members of the Moro-Tissier group are of great importance in dental caries, was corroborated by the studies of McIntosh, James and Lazarus-Barlow.¹ The authors approached the problem of isolation in a way different from that of any of their predecessors. Their aim, as was that of Howe and Hatch, was to get rid of accidental contaminants from the saliva. They first determined experimentally the minimum degree of acidity required to decalcify tooth-structure (extracted, autoclave-sterilized teeth). This they found to be about equivalent to a pH 4. Then, on the assumption that the bacteria which are of prime importance in the decalcification of enamel and dentin must be able to tolerate high acid concentrations, they ultimately inoculated the carious material into broths which were adjusted to reactions around pH 3.5. The bacteria so isolated fall, morphologically into two main groups. They belong to the "acidophilus" group of Moro, and have been named *Bacillus acidophilus odontolyticus*, Type I and II. The terminal acidity produced in glucose broth inoculated with these organisms varied from pH 3.4 to pH 2.2, concentrations

¹ Brit. Jour. Exper. Path., 1922, 138; *ibid.*, 1924, 5, 175.

easily decalcifying enamel. When a tooth, covered with celluloid varnish except for a small area on the enamel, was immersed in a broth culture inoculated with a pure culture of these bacteria "the picture of 'natural' caries was reproduced in every respect; the organisms destroyed the enamel, decalcified part of the dentin and passed down the dentinal tubules for a short distance." Liquefaction foci, indicative of the proteolytic phase of dental caries, are also to be seen in the decalcified dentin. In a control experiment, using *Streptococcus salivarius* in glucose broth, although enamel was destroyed and dentin affected, still no bacteria were found in the tubules and no liquefaction foci were formed.

Rodriguez¹ in a preliminary note reports the constant cultivation of bacteria from deep strata in active dental caries. They apparently belong to Moro's "acidophilus" group and are given the name *Bacillus odontolyticus*. On the basis of morphology they are classifiable into Types I, II and III. It is impossible from the published report to judge their relationship to the findings of McIntosh, James and Lazarus-Barlow.

The Research Laboratories of the Royal Dental Hospital, London,² undertook to investigate (1) the presence of *B. acidophilus odontolyticus* in caries of the enamel, before the dentin was involved, and (2) the presence of this same organism in the carious dentin of deciduous teeth. For the first problem examination was made of 49 permanent teeth. It was found to be a comparatively easy matter to isolate the bacterium in question from caries penetrating deeply into the dentin, but it did not appear to be present in the early stages, *i. e.*, in decay limited to the enamel. In the study of the second problem, 20 deciduous teeth were examined in 6 of which showing advanced involvement of the dentin the organism was not found. Likewise Sierakowski and Zajdel³ did not succeed in isolating this bacterium in 20 per cent of the carious teeth examined. These findings certainly seem to indicate that dental caries can occur in the absence of *B. acidophilus odontolyticus*; thus strengthening the old idea of Miller, Goadby and Kantorowicz that dental caries is not a *specific* bacterial affection.

In a study of 73 cases, Bunting and Palmerlee⁴ found *B. acidophilus* present in 100 per cent (38 cases) of initial caries and in 94 per cent of advanced caries (16 cases). On the other hand

¹ Abstr. Bacteriol., 1923, **7**, 28.

² Brit. Dent. jour., 1923, **44**, 907.

³ Compt. rend. Soc. de biol., 1924, **91**, 961.

⁴ Proc. Soc. Expr. Biol. and Med., 1925, **22**, 296, and Jour. Am. Dent. Assoc., 1925, **12**, 381.

from only 16 per cent of caries-immune mouths (19 cases) was this organism isolated. These authors also describe the artificial production of caries-like lesions of the tooth by *B. acidophilus* in glucose media in from six to eight days when the action is confined to a limited area of the tooth.

Summary.—In looking back over the past few paragraphs it will be seen that the most of the studies on the bacteria of dental caries fall into two groups: (1) that which regards the streptococci as the principal or sole agent in the decalcification of the enamel and dentin, and (2) that which regards representatives of the Moro-Tissier group of acidophilic bacteria as all but exclusively deserving our attention. At the present time, it seems possible to assume one of several attitudes toward these contentions. In the first place we may adhere to one or the other of these two schools of thought, with little qualification. In the second place we can attempt a compromise and at least within ourselves effect it, agreeing with the older view that various bacteria may play a part in dental caries and regarding both the streptococci and the acidophilic bacteria as important factors in the decalcification. The third possibility, the *tertium quid*, is also in the nature of a compromise.

Niedergesaess¹ is apparently the first to propose it and the suggestion originated with Lehmann. In what is essentially a post-script, the view is presented that the *B. necrodentalis* of Goadby may be no other than an organism which has gone under various names. *Aliases* seem to breed trouble whether in man or bacterium. The names to which particular reference is made are the *Bact. guentheri* of the first edition of Lehmann und Neumann's *Bakteriologie*, the *Bac. lactis acidi* of Leichmann, the *Streptococcus lactis* of Kruse, and the *Streptococcus acidi lactici* Grotenfeld of the fourth edition (1907) of Lehmann und Neumann's *Bakteriologie*. If we turn to Goadby's *Mycology* (p. 162) where the morphology of *B. necrodentalis* is described, we find "The bacilli tend to involute rapidly, and form swollen and contorted masses not unlike the streptococcus. In broth cultures the bacilli are very short and appear more like cocci." This certainly is not contradictory to the suggestion of Niedergesaess and Lehmann. Now it has already been remarked that Kligler and Hadley are inclined to align the *B. necrodentalis* with the acidific bacteria of the Moro-Tissier group. We see here a linking up of this latter group with the streptococci, as intermutating or at least confusable organisms.

¹ Proc. Soc. Expt. Biol. and Med. 1925, **22**, 296, and Jour. Am. Dent. Assoc., 1925, **12**, 381.

The edge is somewhat taken off the strangeness of this proposition by the contributions of Goadby and Barrett (1913), Sperling, Clarke, and McIntosh, James and Lazarus-Barlow. Goadby and Barrett in remarking on the frequency of streptobacilli in deep, undecalcified dentin observe that these organisms were once confused with the streptococci but are now regarded as being related to the *necrodentalis* group. Sperling stated that the Gram-positive rods with solely or together with *Streptococcus lacticus* were encountered in the deepest layers of carious dentin, are variants of this streptococcus. Clarke wrote: "When grown on a medium with a neutral or alkaline reaction, *S. mutans* is a non-capsulated coccus . . . ; if, however, the medium becomes acid, it assumes the form of a bacillus." From the second report of McIntosh, James and Lazarus-Barlow is taken the following quotation: "In 61 per cent of the cases in which the organism was isolated it was found in some cultures to resemble a streptococcus so closely in morphology, that it was only after repeated subculture that the organism was finally classified as *B. acidophilus odontolyticus* II." Bunting and Palmerlee¹ emphasize the pleomorphism of their oral strains of *B. acidophilus* and give illustrations (*e. g.*, their Figs. 18 and 19) of both bacillary and streptococcal forms. These expressions permit the surmises, (1) that the cultures described as pure were really mixed, or (2) that all references concern what is really a fairly well-defined group exhibiting marked pleomorphism, or (3) that if the first surmise (1) be correct, at least one member of the mixed culture exhibits pleomorphism. Taking the hint from Hilgers² these surmises might be evaluated by serological methods. It would be interesting and perhaps decisive, to know the serological relations of "streptococci" and of the acidophilic "rods," both isolated from dental caries.

In view of the data presented and of the considerations of the above paragraphs, there seems to be no cogent reason for changing in any essential the older view that the decalcification of the enamel and dentin and the liquefaction of the organic matrix of the dentin may be accomplished by one or more of several bacterial species or groups.

¹ Jour. Am. Dent. Assoc., 1925, **12**, 381.

² Centralbl. f. Bakteriol., I. Abt., Orig., 1924, **93**, 249.

CHAPTER XXI.

THE BACTERIOLOGY OF THE DENTAL PULP.

As has been pointed out in the consideration of the bacteriology of dental caries, infection of the pulp by the passage of bacteria through the dentinal tubuli may occur wherever dental caries involves the dentin. Actual exposure is not necessary and is only the last step of a process long in action. This manner of pulp infection is by far the most frequent and almost the only one of clinical significance. Goadby and Barrett¹ examined the pulps of 20 teeth. Only freshly extracted teeth were used—the subjects of acute pain at the time of removal. The pulp presented the condition of acute pulpitis without gangrene. It was further noted that the tissues surrounding the teeth used were free from inflammation (gingivitis or “pyorrhea alveolaris”). In 10 of the 20 teeth a hard layer of unsoftened dentin existed between the cavity and the pulp chamber. In this group all the cultures were negative, and also no bacteria were seen in direct smears from emulsified pulp tissue. In the other group of 10 teeth the dentin over the pulp was actually softened. In 7 growth of bacteria was obtained. It is to be noted that of 20 pulps examined in a state of acute inflammation sequent to dental caries only 7 showed by culture the presence of bacteria. Henrici and Hartzell² found 43 per cent of positive cultures from vital, grossly normal pulps of teeth showing only dental caries and without gingival or periodontal involvement. These authors³ later reported an histological study of the pulps of 5 teeth showing only dental caries. None was normal; they all showed what the authors regard as in part evidence of bacterial invasion, viz: inflammatory infiltrations, fibrosis, calcification and peculiar fat deposits. The pulps of 42 carious teeth were bacteriologically examined by Collins and Lyne,⁴ 9 of which showed growth on culture. Kelsey⁵ made cultures from the pulps of 75 carious teeth, only 5 of which proved sterile. The pulp is also exposed to infection by traumatic fracture of the tooth or unintentional entrance into the pulp chamber during the preparation of a cavity. These accidents, while analogous to the mode of operation of dental caries, are relatively exceedingly rare.

¹ Loc. cit., 1913.

² Jour. Dent. Res., December, 1919.

³ Ibid., 1920, 2, 557.

⁴ Jour. Nat. Dent. Assn., 1919, 6, 370.

⁵ Brit. Dent. Jour., 1920, 41, 857.

Bacteria can also gain entrance to the pulp sequent to periodontal infection. The pathway followed probably is that of the lymphatic drainage described by Noyes and Dewey.¹ These authors report that the lymphatics of the gingival surface of the gingival trough penetrate into the periodontal ligament, extending toward the root apex. In the periodontal ligament many lymphatics accompany the bloodvessels and nerves, anastomosing at the root apex with lymphatic vessels making their exit from the pulp. Turner and Drew² remark that the "living pulp appears to become readily infected, such infection not necessarily being associated with caries in the ordinary acceptation of the term." Bacteria have been cultivated from vital, grossly normal pulps of teeth without dental caries but with "pyorrhea alveolaris," including even slight degrees of gingivitis. Under these conditions Henrici and Hartzel³ secured 42 per cent of positive results. In the same study as a control all cultures were negative when the tooth was free from both dental caries and periodontal infection. Collins and Lyne⁴ found on culture bacteria present in the pulp of 1 non-carious tooth involved in periodontal inflammation during an examination of 20 such teeth. Kelsey⁵ made cultures from the pulps of 10 sound non-carious teeth, but with "pyorrhea alveolaris." Positive cultures were obtained in 9 (90 per cent). This work has recently received corroboration.⁶ The pulps of 23 "pyorrhetic" teeth were examined, in 19 of which bacteria were demonstrated. In 1 case *B. fusiformis* and spirochetes were found in a section. In order to make the story complete it must be said that Heath,⁷ in testing the findings of Henrici and Hartzel and of Kelsey, examined the pulps of 21 teeth, from only 2 of which bacteria were grown; and even in these 2 instances he is inclined to attribute the positive results to accidental contamination. The technic followed by Heath, however, tends to discredit the value of his negative results. The removed pulps were merely drawn over the surface of a plate of sterile medium. One would expect negative results from such a method except in the case of severely infected pulps. The pulp for such examinations should be thoroughly comminuted in sterile sand moistened with sterile bouillon, and with the mixture of sand, bouillon and pulp sterile media sown. The bacteriological evidence presented in this paragraph for the view that periodontal infection may secondarily

¹ Jour. Am. Med. Assn., October 12, 1918.

² Proc. Roy. Soc. Med., Sect. Odont., 1919, **12**, 104.

³ Loc. cit.

⁴ Loc. cit.

⁵ Loc. cit.

⁶ Brit. Dent. Jour., 1924, **45**, 558.

⁷ Ibid., 1921, **42**, 239.

involve the pulp is borne out by clinical observations. Roy¹ recognizes and describes three types of pulpal trouble associated with "pyorrhea alveolaris:" (1) Dental hyperesthesia; (2) retrogressive pulpitis; (3) trophovascular pulpitis. In previous publications² Roy gives specific histories of such cases. In an histological study of the pulp of 13 teeth without dental caries but with "pyorrhea alveolaris," Henrici and Hartzell³ found 5 normal, 1 showing inflammatory infiltrations, 4 showing fibrosis, 6 showing calcification and 2 showing deposits of fatty tissue of an adult type. These authors regard these changes as in part indicative of bacterial invasion.

Besides infection through the dentinal tubuli and the periodontal lymphatics bacteria may reach the dental pulp from some more or less distant portal by way of the blood stream. The recognition of the possibility of an hematogenous infection is at least as old as Miller's paper, "Introduction to the Study of the Bacteriopathology of the Dental pulp."⁴ It has been suggested that this is the mechanism operative in the pulpal death occurring in the course of such diseases as typhoid fever and influenza. Experimental evidence for this concept has been offered by Beretta.⁵ Guinea-pigs were inoculated intravenously or intraperitoneally with almost avirulent cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens* (*Bacillus prodigiosus*) and *B. clavatus* Biffi. After various intervals of time they were killed with carbon dioxide. They were opened aseptically and with a Pasteur pipette blood was taken from the right heart and small pieces of spleen and bone-marrow. These were planted on ordinary media. The maxillæ and mandibles with the teeth *in situ* were completely removed. The pulp was reached not through the coronal portion of the tooth, but laterally through the alveolus. Pulpal material was inoculated onto ordinary media. It was found that the dental pulp of normal guinea-pigs used as controls at times contained bacteria. *Staphylococcus aureus* injected intravenously remained in the dental pulp of guinea-pigs after its disappearance from the circulation. These organisms were found in the pulp in even larger numbers than in the spleen or bone-marrow. *B. clavatus* likewise was recovered from the pulp after its disappearance from the circulation and in the lymphoid organs it was absent or extraordinarily rare.

¹ L'Odontologie, February 28, 1922.

² English abstracts, Dent. Rec., 1913, **33**, 470; Pacific Dent. Gaz., 1914, **22**, 30.

³ Jour. Dent. Res., 1920, **2**, 537.

⁴ Dent. Cosmos, 1894, **36**, 505; Centralbl. f. Bakteriologie, I. Abt., Orig., 1894, **16**, 447.

⁵ Microbenlokalisationen in der Zahnpulpa auf dem Wege der Blutbahn, Centralbl. f. Bakteriologie, I. Abt., Orig., 1915, **76**, 124.

It is not entirely permissible to project at once upon the plane of human pathology the results of work upon the teeth of guinea-pigs. The teeth of this rodent are rootless or permanently growing with a wide-open apical "foramen" throughout life. For this reason Beretta continued his work upon dogs whose teeth are more comparable to those of man. In contrast to the findings in the guinea-pigs, organisms other than those injected were never encountered in the pulps of dogs. The bacteria used were the *Staphylococcus aureus* and *B. clausii*, injected intravenously. The animals were killed at varying intervals by air emboli. The cultures, taken from the pulp of the canine showed the persistence of the microorganisms in this tissue after their disappearance from the blood.

Although it would be difficult to prove an hematogenous infection of the dental pulp in man the finding in one instance in a histological section by Turner and Drew¹ of diphtheroids and cocci *within the bloodvessels of the dental pulp* is suggestive. A similar observation is reported in the *British Dental Journal* (1924, 45 : 558, Case No. 1115). At all events hematogenous infection of the dental pulp with clinical manifestations must be very rare.

In evaluating the clinical significance of the demonstration of viable bacteria in the living dental pulp, the concept formulated by Adami,² to the effect "that while the tissues of the healthy body are not of necessity free from microorganisms, they are potentially sterile," must be kept in mind. This means that while bacteria may from one cause or another penetrate into the interior of the body, the living tissues under normal conditions are usually able to dispose of them before damage can be done.

THE BACTERIA OF THE DENTAL PULP.

The number of bacteria in a given weight of infected pulp tissue is decidedly less than the number found in the same weight of carious dentin. By reference to the table in the discussion of dental caries adapted from Kligler, it will be seen that a microscopical count of the bacteria from the superficial layer of an exposed pulp is about 37,000,000 per 1 mg.; the figure rises to over 57,000,000 per 1 mg. when the material is taken from the deeper parts of a putrescent pulp. The comparable figure for carious dentin is over 250,000,000. This drop in the number of bacteria is interesting in connection with Miller's occasional observation of

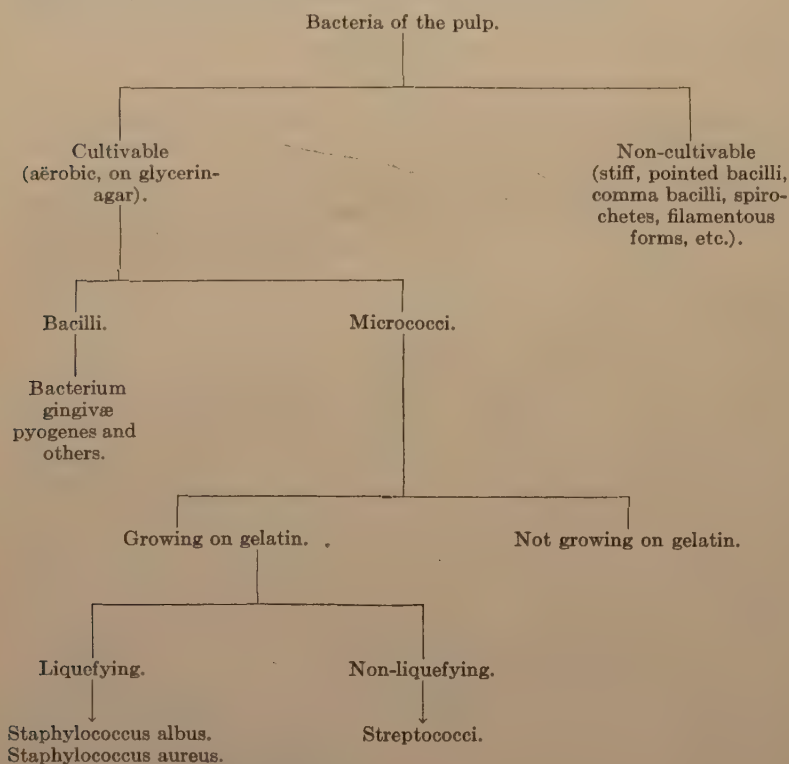
¹ Proc. Roy. Soc. Med., Sect. Odont., 1919, 12, 104.

² Principles of Pathology, 1908, 1, 293.

negative cultures from pulps affected by dental caries and also Goadby's experience that often no bacteria could be microscopically seen or cultivated from pulps which had succumbed to dental caries. Goadby ascribes the pulp involvement in such cases to soluble products of bacterial activity in the dentin, diffusing into the pulp. While this must be a factor, another possible factor for the relatively fewer bacteria in pulp than carious dentin as well as for occasional sterility of affected pulps is to be seen in the vital defensive activities of the highly cellular dental pulp.

Galippe and Vignal¹ isolated various bacterial species from inflamed pulps of carious teeth, including *Bacterium termo* and *Staphylococcus aureus*.

Miller² had found in both purulent and gangrenous dental pulps pyogenic staphylococci and other bacteria, among which was *Bacillus pulpæ pyogenes*. In 1894 Miller³ reported on more intensive studies upon this subject. The following outline, adapted from this publication, gives his views at that time.



¹ L'Odontologie, 1889, 9, 124.

² Die Mikroorganismen der Mundhöhle, 2d ed., 1892, p. 346.

³ Dent. Cosmos, 1894, 36, 505.

Miller attributed little importance to the cultivable species of bacilli as causes of inflammation and suppuration of the pulp. In the latter condition direct microscopical examination of smears always shows small cocci and diplococci, while the putrescent state presents in addition other forms, especially long, stiff, pointed bacilli and threads. Some of the non-cultivable forms and especially the repeated occurrence of spirochetes, are regarded on account of their great numbers in some pulps as probably playing an important role in suppurative processes.

In 50 cases examined culturally the *Streptococcus pyogenes* (probably any streptococcus) was encountered only four times. This relative infrequency is contrary to the findings of many subsequent investigators. From a review of Sieberth's work (see *infra*), Miller¹ had seen no reason up to 1900 to modify essentially his views.

Arkoevy² examined bacteriologically 34 pulps. The following list gives the names of the organisms isolated and the percentage with which each species was found: *B. gangrenæ pulpæ*, 95.34; *Staphylococcus albus*, 18.6; *Staphylococcus aureus*, 34.88; *Staphylococcus citreus*, 4.65; *Streptococcus pyogenes*, 23.25; *B. pyocyaneus* α, 9.3; *B. proteus vulgaris* Hansen, 4.65; *Leptothrix placoides alba*, 2.32; *Staphylococcus cereus albus*, 2.32; *Sarcina aurantiaca*, 2.32; *Sarcina lutea*, 13.95; white yeasts, 4.65; red yeast, 2.32; *Bacterium mycoides roseum*, 2.32; *Micrococcus lactericus*, 6.97; *B. dentalis viridens*, 2.32. Arkoevy's list is conspicuous for the large number of ordinary saprophytes included. The *B. gangrenæ pulpæ*, possibly largely because of the name given to it, experienced some vogue for several years as the most responsible agent for the gangrene of the pulp. It has long since been recognized as a common saprophyte, belonging to the so-called *mesentericus* or "potato" group of bacilli. It is frequently encountered as a contaminant of cultures. It may be found in an exposed gangrenous pulp, as it may be found anywhere else, but there is no reason for regarding it as a causal factor in pulp gangrene. Arkoevy's list calls for two other remarks. *Staphylococcus aureus* was found more frequently than later investigators have found it, and the term "*Streptococcus pyogenes*," as in Miller's work, probably meant any streptococcus.

Zierler³ found what he regarded as the significant organism in pulp gangrene, a sporogenous rod, differing in some respects from

¹ Odont. Bl., Berlin, 1900, 5, 265.

² Experimentelle Untersuchungen ueber Gangraen au der Zahnpulpa und Wundgangraen, Centralbl. f. Bakteriol., 1898, 917.

³ Centralbl. f. Bakteriol., 26, 417.

Arkoevy's *B. gangrenæ pulpæ*, but nevertheless belonging to the "mesentericus" group.

Cook¹ notes that Arkoevy's *B. gangrenæ pulpæ*, Heiner's *Proteus vulgaris*, Babes; *B. proteus littales*, Babes; *B. septicus* and a "bacillus found in putric bronchitis described by Barnabei and Babes," have all been found in pulp gangrene, although the last four mentioned are not regarded by him as the cause thereof. His findings in 40 cases, with the frequency with which each organism was encountered, are given below: *Staphylococcus aureus*, 9; *Staphylococcus albus*, 7; *B. gangrenæ pulpæ*, 34; *Streptococcus pyogenes*, 13; *B. pyocyaneus*, 2; *Sarcina lutea*, 6; *B. dental viridans* (sic), 3; *B. pyogenes fœtidus*, 2; *B. teneus sputigenus*, 3; *B. ulna* (Vignal), 1; tetracoccus, 1. The criticisms applied to Arkoevy's work are equally applicable here.

Sieberth,² in a very important contribution maintained that pulpitis was essentially due to streptococcal invasion. As given in a summary of his dissertation,³ he examined 134 pulps showing all the various pathological states and could find no bacillus, neither Arkoevy's nor Zierler's. In 10 cases no growth occurred. In all other cases streptococci were demonstrated, of which he recognized 8 species (2 long-chained and 6 short-chained). Besides the streptococci, micrococci were found twice and sarcinæ twice.

Preiswerk⁴ isolated bacteria from necrotic or putrescent pulps, using as media gelatin and glycerin agar. The temperature of incubation is given as 23° C. The organisms identified are: *Sarcina alba*, *Streptococcus pyogenes*, *Bacillus vulgatus*, *B. gangrenæ pulpæ*, *B. mesentericus ruber*, *Sarcina pulmomum*, *B. subtilis*, *Leptothrix dichotoma*, *Micrococcus tetragenus*, *Staphylococcus albus*, *Staphylococcus citreus*, *Staphylococcus aureus*, *M. salivarius pyogenes*, *Diplococcus pneumoniae*, white and red yeasts, *B. proteus*, *Vibrio lingualis* (Weibel), *Staphylococcus pyogenes roseus*, *Leptothrix maxima buccalis* (Miller), *Sarcina aurantiaca*, *B. prodigiosus* and *Bacterium zopfii*. This list is furnished by the examination of only 9 pulps. It shows like that of Arkoevy and Cook a very disproportionate number of common, omnipresent saprophytes; a fact making one suspect accidental contamination from saliva, food, water and air.

Goadby⁵ constantly met with streptococci in infected pulps and

¹ Bacteriological Investigation of Pulp Gangrene, Dent. Rev., 1899, 13, 537.

² Die Mikroorganismen der Zahnpulpa, Inaug. Diss., Erlangen, 1900.

³ Centralbl. f. Bakteriol., 28, 302.

⁴ Die Pulpa-Amputation, eine klinische, pathohistologische und bakteriologische Studie, Oesterr.-ung., Vrtijhrschrift. f. Zahnk., 1901, 17, 145.

⁵ Mycology of the Mouth, 1903.

was inclined to think that the ordinary streptococcus of the mouth (*S. brevis*) was the one concerned "rather than the pathogenic streptococcus." He also occasionally found sarcinæ and micrococci. "Staphylococci are not infrequent, the most common variety being *Staphylococcus albus*, although the *Staphylococcus aureus* does also occur . . . *B. necrodentalis* is also often obtainable, as is *B. gingivæ pyogenes*." Goadby also has "noted the constant presence of bacilli of the mesentericus group in pulps . . ." Indeed, ". . . in the bacteriological examination of a large number of dead pulps I have not met with any bacilli with such frequency as the mesentericus group."

Goadby and Barrett¹ examined bacteriologically the pulps of 20 teeth. The teeth had all been freshly extracted on account of the pain of acute pulpitis. The pathological condition of the pulps was that of acute inflammation without gangrene. The supporting tissues of the teeth were unaffected by inflammation (no gingivitis or "pyorrhea alveolaris"). In only 7 of these pulps were bacteria demonstrable on smear or by culture, and these instances were from teeth in which the carious dentin over the pulp chamber was decalcified. In only 1 pulp was a pure culture obtained (*Streptococcus brevis*). The organisms isolated with the number of times encountered are given below: *Streptococcus brevis*, other streptococci, 2; *M. salivarius* (*M. catarrhalis*), 7; streptobacilli, 3; *B. necrodentalis*, 1. No filamentous forms or representatives of the mesentericus group of bacilli were found in these acutely inflamed non-gangrenous pulps.

The next contribution of importance was unique because it applied to anaërobic as well as aërobic cultivation. Monier² examined 5 pulps, most if not all in an at least partial state of gangrene. Aërobic streptococci were recovered 4 times out of 5; *Staphylococcus albus* was recovered once out of 5. In all cases anaërobes were found. The presence of *B. ramosus* was constant; *B. fragilis* was found 3 out of 5 times; the coccobacillus of Veillon and Morax and spirochetes were each found once out of 5. Monier concludes by regarding the anaërobes as of chief importance, conferring the putrescent character upon the process.

Rodella³ likewise emphasized the importance of the anaërobes. He regarded the *Bacillus putrificus* (Bienstock) as dominating the field in the causation of pulpitis, especially of the chronic forms.

¹ Loc. cit., 1913.

² Thèse de Paris, 1904.

³ Ueber anaerobe Mundbakterien und ihre Bedeutung, Arch. f. Hyg., 1905, 53, 329.

In 1909 Mayrhofer¹ studied diseased dental pulps in 152 cases, limiting his attention to the pyogenic microorganisms. Streptococci were found in 96 per cent of the 152 pulps. In 70 per cent these were the only organisms cultivated. The other forms isolated were rods, staphylococci and yeasts. Mayrhofer regards that streptococci are the most important agents and even is inclined to the opinion that no pulp gangrene can occur without streptococci.

Sommer² studied the bacteriology of 12 infected, necrotic pulps. He limited his material to such teeth as showed a more or less thick layer of softened dentin still over the pulp chamber, *i. e.*, without gross exposure. The inoculation of media was done at once after extraction, and cultivation was made both aërobically and anaërobically. Direct smears from the contents of the pulp chamber were also microscopically examined. The media were agar, glucose agar and glucose agar plus blood serum or ascitic fluid. The organisms isolated were *B. fusiformis*, *Granulobacillus* *sp.* (Sommer notes that this organism may be identical with the organism described by Rodella under the same name and also with the latter's *B. putrificus* (Bienstock)), *B. ramosus*, *B. perfringens*, possibly an anaërobic streptococcus, an aërobic streptococcus agreeing with that described by Sieberth as A and by Kantorowicz as a, several closely related species of rods agreeing with forms described by Kantorowicz and *B. mesentericus* (repeatedly).

From these 12 pulps Sommer made 109 anaërobic cultures, with the following results: *B. fusiformis* was encountered 67 times; *Granulobacillus* *sp.*, 34 times; *B. ramosus*, 3 times; *B. perfringens*, 5 times. The beginning of the gangrenous process in the dental pulp is accompanied, in the opinion of Sommer, by a striking increase in the number of fusiform bacilli. With the passage of time the numbers of the granulobacillus seem to increase at least relatively more rapidly. Such anaërobic forms as these seem to constitute the major part of the flora in the cases under consideration. Spirochetes were not cultivated. In a large number of smears examined they did not seem to constitute an important factor in the process of pulp gangrene as it occurs in unexposed pulps.

Kliger³ in his very valuable researches observed that decay of the dental pulp is a process that is different in character from decay of enamel and dentin. Associated in this process are: (a) Relatively low bacterial count; (b) drop in the relative number

¹ Prinzipien einer rationellen Therapie der Pulpagangraen und ihrer haeufigsten Folgezustände, 1909, Fischer, Jena.

² Deutsch. Monatschrft. f. Zahnk., 1915, 33, 297.

³ Jour. Allied Dent. Soc., 1915, 10, 313.

of cocci, similar to that found in primary decay, but differing from the latter also in showing a disappearance of the thread forms; (c) presence in all cases of anaërobic putrefying bacilli. The acidific type (*B. necrodentalis*, *B. acidophilus*) persists in practically the same abundance as in the initial stages of decay. There is no doubt that the anaërobic spore-formers, which possess powerful proteolytic properties and are found in decay of the pulp and under no other condition, might be the agents concerned in initiating and completing this process. Kliger's results in this connection seem to confirm in a striking manner Rodella's conclusion that the anaërobic, putrefying bacillus (*B. putrificus*, Bienstock) is closely related to the process of pulp decay.

Turner and Drew,¹ by direct microscopical examination (no cultures) found in the living pulp various types of diphtheroids, streptococci, spirochetes and staphylococci. The mixed infections were always associated with dental caries.

Henrici and Hartzell² examined culturally 115 clinically vital, grossly normal pulps, in which are included the pulps from 22 teeth free of dental caries and "pyorrhea alveolaris." From this latter group no growth was obtained. The medium was suitable for both aërobic and anaërobic organisms. The following species were found and the number of times encountered is also given. *Streptococcus viridans*, 29; *S. hemolyticus*, 1; *Staphylococcus albus*, 8; *Staphylococcus aureus*, 1; diphtheroid bacilli, 3; *B. proteus*, 1; *B. coli*, 1. From 5 specimens 2 varieties were isolated: viz: *Streptococcus viridans* and *Staphylococcus albus*, 2; *Streptococcus viridans* and diphtheroid, 1; *Streptococcus viridans* and *Bacillus proteus*, 1; *Staphylococcus albus* and a diphtheroid, 1. The pre-eminence of the alpha type of streptococci under these conditions is evident. The importance of this report lies in the fact that the pulps examined were in a very early stage of involvement, being grossly, clinically normal. The findings are practically identical with those of Turner and Drew (see *supra*), making allowance for the fact that the cultural conditions would not permit of the development of spirochetes.

Kelsey³ in a cultural examination of 125 pulps found the following types together with their respective frequencies: Streptobacilli, 71.2 per cent; streptococci, 48.8 per cent; staphylococci, 37.6 per cent; tetracocci, 12 per cent; Gram-positive bacilli, 10.6 per cent; Gram-negative bacilli, 7.2 per cent; cocci, 7.2 per cent; diplococci, 4.8 per cent; filamentous forms, 3.2 per cent.

The attempt to correlate the data offered in the above para-

¹ Loc. cit., 1919.

² Loc. cit., 1919.

³ Loc. cit., 1920.

graphs is complicated by the fact that it is usually impossible from the original reports to form any reliable idea regarding the condition presented by the pulp examined.

A consideration which will perhaps give the clue to the relation of bacteria to some of the pathological states of this organ is the peculiar situation of the dental pulp, enclosed by hard, unyielding dentin. Most tissues or parts increase in volume during acute inflammation, as a result of the vascular exudation. When the possibility of swelling is very limited or *nil*, as in the case of the central nervous system or in the dental pulp, the vitality of the part is most precarious. In the case of the pulp the accumulating, intercellular exudate compresses the bloodvessels and impairs the nutrition of the organ by interfering with the ingress of food and oxygen and with the carrying away of harmful metabolites. This often results in the rapid death of the pulp, *i. e.*, necrosis or gangrene.

Certain bacteria and in the case of dental caries, probably also the diffusible products of the activity of any organism, reaching the pulp, call forth the process of inflammation. If, and when, necrosis results—particularly sequent to a state of passive hyperemia which favors moist gangrene—then almost any saprophyte which is adaptable to the conditions obtaining in the dead organ, and which chance brings to that organ, may contribute a share to its dissolution, and may be isolated from the pulpal débris.

The streptococci on account of their invasive properties would seem to be the bacteria best fitted for inducing the initial inflammation in the pulp. The acidific bacteria (Moro-Tissier) possess little or no invasive power, although their products might initiate the inflammatory reaction. The almost constant finding of streptococci by Henrici and Hartzell in clinically normal pulps supports the view that these microorganisms very early gain access to the pulp. They thus have the opportunity and they also have the natural endowments to act as irritants and inflammatory stimuli. If, as a result of this inflammation, the pulp succumbs, then non-invasive types of putrefactive saprophytes can survive and flourish in the necrotic tissue as they would in any dead organic animal material. Some of these saprophytic types would outgrow the others and be isolated on culture with a greater frequency. As a matter of fact, Arkoevy and Zierler have emphasized the presence of members of the aërobic and facultative anaërobic “mesentericus” group, and Goadby has also noticed the constant presence of such organisms. On the other hand, Rodella, Sommer and Kliger have emphasized the importance of the strictly anaërobic putrefactive organisms, *B. putrificus*, to wit.

CHAPTER XXII.

THE BACTERIOLOGY OF PERIAPICAL INFECTIONS.

BACTERIA may gain access to the periapical tissues, as also in the case of the dental pulp by way of: (1) The lymphatic drainage of the periodontal ligament; (2) the blood or lymph circulation from more remote parts; (3) an infected dental pulp sequent on dental caries or fracture. The third pathway accounts for by far the greatest number of periapical infections. At least as early as 1879 or 1880, E. T. Darby called attention to the paradox of abscesses on teeth with living pulps. Kirk¹ pointed out in 1900 the periodontal origin of this condition, *i. e.*, that the bacteria in such "abscesses" had not come by way of the pulp but by way of periodontal lesions. This view has been corroborated by Roy.² Hartzell³ notes that during a two-year period he has recorded 27 "abscesses" at the apices of teeth with vital pulps. In most if not all of these cases it is likely that the bacteria followed the first or second rather than the third pathway noted above. Meisser and Rosenow aseptically removed healthy pulps from the teeth in dogs and filled the canals without resorting to irritating chemicals. Cultures taken at this time from the root canal and from the periapical region gave negative results. They then introduced into the pulp chamber of another tooth in the same animal a pure culture of a streptococcus identifiable by its known predilection for localizing in certain organs of the rabbit. After a lapse of time this streptococcus was in some instances recovered from the periapical tissues of those teeth whose canals had been aseptically filled. This fact indicates an infection of hematogenous or lymphogenous origin.

The clinically significance of the observations of Kirk, Hartzell, Roy and of Rosenow and Meisser is important. They show that bacteria are constantly being brought to the periapical region. As long as the local resistance of that part is high, Adami's concept of the potential sterility of healthy tissues holds good and no harm is done. When, however, the local resistance is lowered from any cause, *e. g.*, "devitalization" and root-canal filling or possibly also

¹ Pericemental Abscesses, *Dent. Cosmos*, 1900, **42**, 1149.

² *Dent. Cosmos*, July, 1918.

³ *Dent. Review*, 1916, **30**, 108.

occlusal trauma, the bacteria arriving may gain a foothold and initiate genuine pathological changes. These considerations teach us why we should hesitate to "devitalize" a healthy pulp and why periapical infection tends to persist or to recur in spite of the most intelligent and conscientious efforts. The "root-canal" problem, which rather is essentially the *problem of periapical infection*, is not limited to the single tooth under treatment, but calls for synchronous attention to the periodontal condition of that tooth and adjacent teeth and to the periapical condition of the other teeth in the mouth. Frequently, we have found continued positive cultures from a tooth which did not become negative until attention had been given to other pulpless teeth in that mouth. Then it was often seen that several teeth were mutually involved and that as long as the cultures from one remained positive they all would be positive and that when the culture from one became negative the cultures from all proved negative. Severe or extensive periodontal or periapical infection in a mouth will complicate the problem of the treatment of the periapical region of any particular tooth. Even though apparently satisfactory results be obtained, the possibility of reinfection, probably by way of the lymphatics, is likely sooner or later to be realized.

THE BACTERIA OF PERIAPICAL INFECTIONS.

Haussmann¹ microscopically examined the pus from an abscess on one of his own teeth. He found non-motile bacterial filaments, the "gliococcus" and numerous isolated cocci. The cocci also appeared in short chains but no long chains were found. The coccal forms were identified with "coccobacteria septica." It is impossible to refer either the gliococcus or the coccobacteria septica to definite bacterial forms known today. Billroth, who introduced these terms in 1874, was working with mixed cultures. It is certain, however, that Haussmann saw the ordinary short-chain streptococcus.

Nannotti² found what he regarded as the pneumococcus in two abscesses on carious teeth and in one mandibular abscess.

Schreier³ made cultures from the periapical region of 15 infected teeth. The organism most frequently isolated (11 times altogether, 6 of which in pure culture) was identified as the pneumococcus. It

¹ Berl. klin. Wehnschr., 1878, **15**, 191.

² Lo Sperimentale, 1891, **45**, No. 12.

³ Oesterr.-ungar. Vrtljhrschrt. f. Zahnk., 1893, **9**, 133.

is most likely that the bacterium in question is not what is known today as the pneumococcus, but that Schreier had found the *Streptococcus viridans*. The possibility of this same confusion of an alpha streptococcus with a pneumococcus must be kept in mind in evaluating all of these reports. *Staphylococcus albus* was isolated from 7 of the 15 cases 3 times in pure cultures. The only other organism found was the *Staphylococcus aureus* (in 2 cases, in 1 of which in pure culture).

Roughton¹ isolated *Streptococcus pyogenes* from an apical "abscess." It is to be remembered that by his term he probably refers to a streptococcus but not necessarily to the species recognized today as *pyogenes*.

Arkoëvy (1898) found his *B. gangrenæ pulpæ* (a member of the "mesentericus" group of saprophytes) in 3 out of 4 cases of periapical infection. In 2 of the 3 instances it was associated with both the *Staphylococcus aureus* and the *Staphylococcus albus*. No growth appeared on the culture from the fourth case.

The observation of Kirk² of pneumococci and streptococci in the "abscesses" on teeth with vital pulps more properly belongs to the description of the bacteriology of "pyorrhea alveolaris."

Goadby³ found *Staphylococcus aureus* relatively rarely (3 of 20 cases examined), *B. coli*, 2; *Staphylococcus albus*, rather frequently; *M. tetragenus* and yeasts, but implies that one organism, named by him the *Staphylococcus viscosus* is of special importance because of the frequency with which it has been isolated in pure culture. Goadby also refers to the presence of strict anaërobes, but has been unable to isolate them in pure culture.

In 1904 Monier⁴ reported on the bacteriological examination of periapical "abscesses" sequent to dental caries. His work is of special interest because of the emphasis he layed on anaërobic technic. Aërobic cultures twice remained sterile. The findings of the examination of smears made directly from the pus run as follows: Micrococci and a bacillus; a few micrococci, a Gram-positive bacillus and numerous small Gram-negative bacilli; delicate filamentous forms and fusiform bacilli. The presence of a streptococcus was quite constant, being isolated 4 times out of 6 cases; in fact it was the only organism isolated under aërobic conditions. On the other hand, strict anaërobes were always present: *B. ramosus* in every case, 6; *B. fragilis*, 3; the coccobacillus of Veillon

¹ Trans. Odont. Soc. Great Britain, 1893, 25, 71.

² Dent. Cosmos, 1900, 42, 1149.

³ Mycology of the Mouth, 1903.

⁴ Thèse de Paris.

and Morax, 1; a previously undescribed filamentous bacillus, 2. Monier concludes that the strict anaërobies are most important in the production of periapical infection, and that to these is attributable the putrescent character of the exudate.

Vincent,¹ in describing the pyogenic function of the fusiform bacilli and spirochetes acting together, notes that he found these microorganisms 7 times out of 17 cases of dental infections; twice they were in pure culture and in the other 5 cases mixed with streptococci, staphylococci and strictly anaërobic bacilli. Davis and Moorehead² examined bacteriologically 20 cases of periapical infection. Careful attention was given to prevent accidental contamination. Streptococci and fusiform bacilli were found in all. Sixteen cases in addition revealed spirochetes. (From the illustration in the original article one would judge that these belong to the *Spirochaeta vincenti*.) These authors conclude that periapical infections usually result from the combined activities of these three types of microorganisms.

In a very interesting study Idman³ made an important contribution to our limited knowledge of the anaërobic flora of periapical infections. His results are summarized in the following table.

	CASES.							
	A.	B.	C.	D.	E.	F.	G.	H.
Aërobic or facultative forms:								
Streptococci	+	+	..	+	..	+	+	
Staphylococci	..	+	..	..	..	..	+	+
Diplococci	..	+						
M. tetragenus	..	+						
B. mesentericus	+							
Diphtheroid	..	+						
Other rods	..	..		+	+			
Obligate anaërobic forms:								
Streptococci	+	..	+	+	..	..	+	
Staphylococcus parvulus	..	..	..	+	..	+		
Staphylococcus jungano	..	..	..	..	..	+		
B. ramosus	+	+	+	+	+	+	+	+
B. thetoides	..	..	+	+	..	+		
B. perfringers	..	..	+	+				
B. fusiformis	..	..	..	..	..	+		
B. bifidus communis	..	..	..	+				
Other rods	+	..	..	+				

These results of Idman on the whole serve to corroborate the findings of Monier. Aërobic and anaërobic forms were both present

¹ Compt. rend. Soc. de biol., 1905, 58, 772.

² Jour. Dent. Res., 1923, 5, 1.

³ Bakteriologische Untersuchungen von im Anschluss an Pulpitis purulenta und Gangraena pulpæ auftretenden periostalen Abszessen mit besonderer Berücksichtigung der obligat anaeroben Mikro-organismen, Arb. a. d. path. Inst. d. Univ. Helsingfors (Finland), 1913, Fischer, Jena.

in every case with the latter types predominating. The anaërobic *B. ramosus* was encountered with invariable constancy, while next in frequency were the aërobic and anaërobic streptococci. Idman is convinced that the peculiar character of the periapical infections is determined, at least chiefly, by the anaërobic bacteria.

In 1919 Head and Roos¹ gave a preliminary report on their bacteriological findings of 130 specimens from 100 cases of periapical infection. The isolations were made anaërobically, but it is impossible to be sure that all the organisms isolated were strict anaërobes. Streptococci, with but few exceptions belonging to the *Streptococcus viridans* group, were present in 124 specimens. In 10 instances they were in pure culture. Members of the Moro-Tissier group were found in 45 specimens. These authors, however, are inclined to lay the major stress upon a minute, strictly anaërobic, Gram-negative "coccobacillus" which was found in 90 specimens from 90 different cases.

The bacteriological findings in periapical infections, presumably obtained by aërobic technic only, were reported by Mayrhofer.² Thirty-eight specimens were taken by way of the root canals, with the following results: Streptococci in pure culture, 14 times; streptococci and rods, 7 times; streptococci and staphylococci, 9 times; streptococci, staphylococci and rods, 6 times; only rods, 2 times. Thus streptococci were found 36 times out of 38 cases of periapical inflammation; Mayrhofer considers these forms to be by far the most important bacterial factor in this condition.

In a study of the bacteriology of alveolar "abscess" and infected root canals of over 40 cases, Gilmer and Moody³ obtained many graded variations from a hemolytic streptococcus, with a wide zone of hemolysis in the acute abscesses, to a *Streptococcus viridans* in the chronic, and once a *Streptococcus mucosus* was the predominating organism. These organisms, particularly the green-producing streptococci, will in many instances grow as well anaërobically as they will aërobically. The anaërobic cultures which do contain streptococci are rarely pure. Some contain *Bacillus fusiformis* in varying numbers, sometimes even in practically pure culture. Aërobically isolated colonies of *Staphylococcus aureus* or *albus*, *Micrococcus catarrhalis* and some unidentified saprophytic organisms are occasionally obtained. In two instances a diphtheroid bacillus was found which grew under both aërobic and anaërobic

¹ Jour. Dent. Res., 1919, vol. 1.

² Prinzipien einer rationellen Therapie der Pulpagangraen, Fischer, Jena, 1909.

³ Jour. Am. Med. Assn., 1914, 63, 2023.

conditions. Anaërobically in old cultures, in material from three different abscesses a black pigment-producing organism developed. This organism is slow growing and does not usually appear for about five days. Gilmer and Moody do not believe that it is of any importance in these infections.

Later Moody¹ reported on the periapical infections in 55 patients. Four of the cultures showed no growth, 2 were discarded because of recognized contamination and the isolations from 49 cultures furnished the basis for his conclusions. Briefly stated these are that aërobic methods yielded in nearly every instance pure cultures of *Streptococcus viridans*, but that stray colonies of staphylococci and hemolytic streptococci might occasionally be encountered.

Hartzell and Henrici² summarize their experiences by stating essentially that streptococci are constantly present in "apical abscesses."

In periapical infections Ulrich³ found a great preponderance of *Streptococcus viridans*. Among the other organisms isolated were *S. hemolyticus*, *S. mucosus*, *Staphylococcus aureus*, *Staphylococcus albus* and *M. catarrhalis*.

Thoma⁴ studied the bacteriology of periapical "abscesses." Aërobic methods were used routinely, but anaërobic cultivations were also at times made. The bacteria found are the following: Streptococci, growing in the majority of cases both aërobically and anaërobically, sometimes in pure culture, but more frequently mixed with *Staphylococcus aureus* or *albus*. Besides these common pyogenic bacteria he found an admixture of many other pathogenic and saprophytic bacteria, such as the fusiform bacillus, *B. coli*, the influenza bacillus (Pfeiffer), *B. proteus*, the pneumococcus and others.

In 1917 Mendel⁵ mentioned the isolation by anaërobic methods of a Gram-positive bacillus very similar to the diphtheroid of Jungano from a periapical abscess.

Collins and Lyne⁶ cultivated from the apical tissues of infected teeth: *B. fusiformis*, *Streptococcus viridans*, *Streptococcus hemolyticus* and spirochetes.

Smith and Ludwick⁷ limited their attention to the bacteriology of the "abscessed" teeth of children. One hundred and seven cases

¹ Jour. Infect. Dis., 1916, **19**, 515.

² Jour. Am. Med. Assn., 1915, **64**, 1055.

³ Ibid., **65**, 1619.

⁴ Jour. Allied Dent. Soc., 1916, **11**, 95.

⁵ Compt. rend. Soc. de biol., 1917, **80**, 962.

⁶ Jour. Nat. Dent. Assn., 1919, **6**, 370.

⁷ Nebraska State Med. Jour., 1919, **4**, 131.

were examined; 8 showed no growth. The names of the bacteria and the number of times each was isolated follow: *Streptococcus hemolyticus*, 26; *Streptococcus pyogenes*, 19; *Streptococcus viridans*, 2; *Staphylococcus citreus*, 9; *Staphylococcus aureus*, 36; *Staphylococcus albus*, 7; *Pneumococcus pyocyanea*, 1; *Diplococcus pneumoniae*, 18; *M. catarrhalis*, 4; *B. fusiformis*, 1; Vincent's spirochete, 1; a diphtheroid, 3.

The bacteriology of the periapical tissues of pulpless teeth and of teeth with living pulps was studied by Lucas.¹ In his summary it is impossible to separate the two categories of teeth. The organisms identified and the number of times each was isolated are given in the following table. In all 181 specimens were examined, growth not appearing on 26 of them.

<i>Streptococcus viridans</i>	11
<i>S. pyogenes</i>	37
<i>S. hemolyticus</i> (in 4 per cent of examinations)	
<i>Pneumococcus</i>	30
<i>M. catarrhalis</i>	6
<i>Streptobacilli</i>	5
Gram bacilli (sic)	11
<i>B. subtilis</i>	9
Vincent's organisms	8
Diphtheroids	10

Lucas regards *B. subtilis* as a contaminant, probably with right. What he means by his "Gram bacilli" is problematical. In his text it appears that the possibility that they are the *B. prodigiosus* has been considered. If they resemble that organism I am of the opinion that they are contaminants. I am also of the opinion, based on the context, that from a critical standpoint it would be wise to refrain from accepting the specific identifications of the streptococci, but to group such organisms together with the "pneumococci" under the non-committal term, streptococci.

Berwick² studied in 71 cultures the bacteria of periapical, radio-lucent areas. Three hemolytic streptococci were isolated while non-hemolytic streptococci were found in pure culture 22 times. Staphylococci were in pure culture 8 times. Two unidentified anaërobes were encountered. A large number of the cultures showed mixed infection, and often two or more different strains of streptococci were isolated from the same infected area.

Fraser,³ from cultures of the apices of 120 extracted teeth, supplied by 82 patients, obtained the following results:

¹ Dent. Summary, 1920, **40**, 955.

² Jour. Inf. Dis., 1921, **29**, 537.

³ Brit. Dent. Jour., 1923, **44**, 1350.

Streptococcus viridans in 108 cases, or 90 per cent; streptococci were recognized in smears but not isolated in 12 cases. Pure cultures of *Streptococcus viridans* were obtained in 45 cases, or 42 per cent. From 68 mixed cultures the following were obtained:

Streptococci with:	
Staphylococcus aureus	11
B. albus	22
M. catarrhalis	11
S. aureus and M. catarrhalis	8
S. albus and M. catarrhalis	4
Gram-negative bacilli	2
S. aureus and M. catarrhalis	1
Streptococcus viridans and a beta streptococcus	2
Streptococcus viridans with:	
Alpha prime type and Staphylococcus albus	1
Alpha prime streptococcus	4
Beta streptococcus and M. catarrhalis	1

In brief, the results obtained may be summarized as follows:

Streptococci present in 120 cases, or 100 per cent; *Streptococcus viridans* present in 108 cases; beta type streptococci present in 3 cases; alpha prime streptococci, 6 times; *Staphylococcus aureus* present in 20 cases; *Staphylococcus albus* present in 27 cases; *M. catarrhalis* present in 24 cases; Gram-negative bacilli present in 3 cases. Fraser believes that the primary infecting organism in all cases of periapical abscess to be *Streptococcus viridans*.

Broderick¹ bacteriologically examined the apices of 100 periapically injected teeth. The cultures were made from the apical surface of the tooth, after extraction and incubated aërobically. He found in every one of the 100 instances streptococci. No record is given of any other organism isolated. The streptococci (in all 131 strains) were classified according to Holman (in the discussion of the *Streptococcus-pneumococcus Group*) on the basis of hemolysis and fermentative powers. The frequency and associations of the various strains is given below. *S. salivarius* occurred 45 times, 26 times being the only variety present. It was associated with:

S. mitis	9
S. ignavus	6
S. equinus	2
S. angiosus	1
With S. mitis, S. ignavus and S. equinus	1

S. mitis occurred 32 times, 16 times being the only variety. It was associated with:

S. salivarius	9
S. equinus	2
S. ignavus	4
With S. salivarius, S. equinus and S. ignavus	1

¹ Brit. Dent. Jour., 1924, 45, 1301.

S. ignavus occurred 26 times, 12 times being the only variety. It was associated with:

<i>S. salivarius</i>	6
<i>S. mitis</i>	4
<i>S. equinus</i>	1
With <i>S. salivarius</i> , <i>S. mitis</i> and <i>S. equinus</i>	1
With <i>S. equinus</i> and <i>S. angiosus</i>	1
With non-hemolyticus I	1

S. equinus occurred 11 times, 4 times being the only variety. It was associated with:

<i>S. salivarius</i>	2
<i>S. mitis</i>	2
<i>S. ignavus</i>	1
With <i>S. salivarius</i> , <i>S. mitis</i> and <i>S. ignavus</i>	1

S. angiosus occurred 6 times, 4 times being the only variety. It was associated with:

<i>S. salivarius</i>	1
With <i>S. equinus</i> and <i>S. ignavus</i>	1

S. faecalis occurred 6 times, in each case being the only variety.

S. subacidus occurred 3 times, in each case being the only variety.

S. non-hæmolyticus I occurred twice, once being the only variety and once being associated with *S. ignavus*.

Before passing on it is well to point out that only 9 of the strains isolated were hemolytic, while 121 strains were characterized as non-hemolytic. None of the strains fell into Holman's groups, *S. non-hæmolyticus II* and *S. non-hæmolyticus III*. Representatives of these two groups were also absent among the streptococci isolated from oral lesions by Glynn and Digby, by Goadby and by Hartzel. This study only lends further confirmation and emphasis to what has long been known, namely, that numerous streptococcal strains are present in periapical infections and that these strains are not at least as yet distinguishable from those habitually found in the normal saliva or in periodontal inflammations.

Summary.—Knowing that most instances of clinically significant periapical infection arise by invasion from the dental pulp, the similarity between the flora of the pulp and the periapical tissues is not surprising. It is also true that no distinction on the basis of the bacteria present can as yet be drawn between the periapical infections due to extension from the pulp and those due to extension through the alveolodental periosteum or to other lymphogenous or hematogenous origin. On the whole the number of species of

bacteria isolated from the inflamed periapical region has probably been less than the number described for the gangrenous pulp or for the pockets of "pyorrhea alveolaris." This finds a ready explanation in the exposure of the two latter sites to accidental contamination with saprophytes, while the deeper and more secluded periapical region is in the nature of things more protected against the intrusion of transitory saprophytes and such of these organisms as gain access are more readily disposed of by the inflammatory reaction.

Of the microorganisms that are either aërobic or possess the ability of living under either aërobic and anaërobic condition the common pyogenic cocci are encountered with the greatest frequency. Of these cocci the streptococci by far outnumber all other types, and the vast majority of the streptococci are members of the short-chained, *alpha* or so-called *viridans* group, characterized pathogenically by a relatively low virulency. The *alpha* streptococci, isolated from periapical or, for that matter, from any oral infection, do not constitute a single well-defined entity on the basis of fermentation reactions or serological tests. Judged by these criteria the streptococci of oral infection fall into many separate groups, and are not distinguishable from the *alpha* streptococci from the saliva, from the tonsils or from other localities, diseased or healthy, of the human body.

Aërobic or facultative diphtheroids are also encountered in periapical infections. They have never been systematically studied. Until their frequency, types and functions are well established, our knowledge of periapical infection will be far from the completeness desirable.

Almost the same thing can be said of the strict anaërobes. In spite of the excellent and suggestive studies of Monier and Idman, which show anaërobes are almost, if not quite, invariably present, routine examinations continue to ignore these microorganisms.

Probably no discussion of periapical infection would be satisfactory today without some reference to the roentgenographical findings. In the first place most periapical, radiolucent areas are found on examination to be infected. It must also be remembered that infection may be present even in the absence of all roentgenographical evidence. This is especially so in acute cases. Some time is necessary for the changes in the bone to become objectively demonstrable. Furthermore, roentgenographical evidence of bone regeneration after treatment does not of itself justify the conclusion that the periapical region has been sterilized and is sterile.

Osteogenesis will occur in the presence of persisting infection. On the other hand, radiolucent areas do *not* invariably mean infection. They may occur about the apices of teeth with living pulps and with non-infected (potentially) sterile periapical tissues, *e. g.*, in the case of teeth subjected to occlusal trauma. Berwick¹ was unable to correlate the various appearances on the roentgenographical film with any particular type of infecting bacterium, *i. e.*, infection with a beta streptococcus does not determine changes in the alveolar bone, which are roentgenographically distinguishable from the changes determined by, let us say, the *Streptococcus viridans*. Rosenow,² in summarizing some of the work in his laboratory by Meisser, says "That the roentgen-ray-negative tooth may harbor streptococci that have a higher degree of specific invasive power than those isolated from pulpless teeth in which marked rarefaction is apparent."

¹ Jour. Infec. Dis., 1921, **29**, 537.

² Jour. Am. Dent. Assn., October, 1924.

CHAPTER XXIII.

PERIODONTAL INFECTION: THE BACTERIOLOGY OF "PYORRHEA ALVEOLARIS."

IN the present discussion and throughout this book this term is used to designate any pathological condition which presents as a symptom more or less pocket formation around a tooth together with a flow of pus from that pocket. Taken in its literal, etymological sense, and as a symptom rather than a pathological entity, the phrase "pyorrhea alveolaris" is not subject to controversy. As a matter of fact, although this type of pyorrhea is invariably indicative of infection, this infection itself is probably always secondary to fundamental, indispensable changes in the supporting tissues of the tooth, which are not primarily infectious in nature. Talbot, Hopewell-Smith, Roy and Gottlieb and Fleischmann have shown that the infection is preceded by an atrophy of the free margin of the alveolar process. More recently Box has described a proliferative, inflammatory change in the periodontal ligament, which soon causes a rarefaction of the alveolar bone. Either an atrophy or the "rarefying pericementitis fibrosa" of Box would by destruction of the bony scaffolding supporting the gingivæ around the tooth permit the falling away of these gingivæ from the tooth unless they are absorbed *pari passu* with the alveolar destruction. Their separation from the tooth would at once create a pocket. In this would occur stagnation of the saliva and the collection of food débris—conditions both favorable for bacterial growth. It must not be forgotten also that an antecedent marginal gingivitis, whatever be its "cause" (neglect, salivary calculus or the traumatic insults of poorly finished gingival margins of fillings, of poorly adapted bands of crowns or bridge abutments, or of incorrectly adjusted partial dentures, or from food impaction onto the interdental papillæ when approximal fillings do not restore adequately the surfaces of contact, etc.), has very commonly been observed, and would by direct extension accelerate the infection of the pocket.

While we regard the infection which manifests itself as a pyorrhea alveolaris as at least usually *secondary* to certain non-infectious changes in the supporting structures of the tooth, we do not wish to give countenance to the view that the infectious phase of peri-

odontal disease is of small consequence. Whenever infection is present (and it is present whenever we have a pyorrhea alveolaris, *i. e.*, a flow of *pus* from the alveolus), it complicates and aggravates the condition and to it almost alone is due the systemic import of periodontal disease.

If we accept the viewpoint put forth in the above paragraphs as to the pathogenesis of "pyorrhea alveolaris," our concept of the problem will become clearer and the significance of some laboratory and clinical findings will be better appreciated. In the first place, if this condition or group of conditions is not primarily infectious in nature it becomes very unlikely that its infectious phase is determined by a single, specific microorganism. Much time has been spent in the search of the microbic cause of "pyorrhea alveolaris," and as we shall see no such cause has been found. In the second place, if the infectious phase of these conditions be not primary then it is illogical to expect satisfactory or permanent results from treatment directed primarily or exclusively against the microbes of the pockets. No single therapeutic agent or method will be effective, but to be rational due regard must be given to the several factors operative.

THE MICROORGANISMS OF "PYORRHEA ALVEOLARIS."

If we consider the taxonomic position of the spirochetes as unsettled then the only indubitable protozoan species to which has been ascribed an important role in these conditions is the *Entamoeba gingivalis* (Gros, 1849). In 1914 Barrett¹ found this organism in the pyorrhetic pockets of 46 individuals examined, but not in the normal mouths of 7 other individuals. This fact, together with the observed clinical improvement following the use of emetin hydrochlorid (used with universal success in amoebic dysentery and regarded then as a specific amoebicide) was regarded by him as strongly favoring the view that this protozoön exerted a real pathogenic influence. About the same time Bass and Johns² came in the same way to essentially the same conclusion.

The organism in question varies in size from 4 μ to 60 μ , but usually from 10 μ to 20 μ in diameter. Ectoplasm and endoplasm are fairly sharply differentiated. Pseudopodia are well developed; lobose, typically rounded, not pointed. The nucleus cannot, or at

[¹ Dent. Cosmos, August, 1914.

² New Orleans Med. and Surg. Jour., 1914, 67, 456; Jour. Am. Med. Assn., February 13, 1915, p. 553.

best very indistinctly, be seen in the living animal. In properly fixed and stained specimens the nucleus is spherical and vesicular, $2.5\ \mu$ to $3\ \mu$ in diameter, appearing as a very definite ring in optical

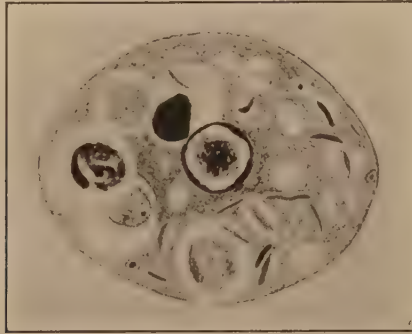


FIG. 83.—*Entamoeba gingivalis* containing partly digested nuclei of leukocytes and various rod-shaped bacteria. Remnants of food contents in the smaller vacuoles. No ectoplasm. (Kofoid and Swezy.)

section. It contains a spherical karyosome, either eccentrically or centrally placed. The endoplasm contains usually numerous food vacuoles, enclosing peculiar ingested bodies staining intensely with



FIG. 84.—*Entamoeba gingivalis* with retracted pseudopodia, peripheral ectoplasm, three leukocytic food vacuoles. (Kofoid and Swezy.)

nuclear dyes. Some of these inclusions may be erythrocytes, but most of them are probably the remains of nuclei either of salivary "corpuscles" or of other leukocytes or of other cells. Bacteria are

also abundantly present in these food vacuoles. In common with other parasitic rhizopods, no pulsating vacuole is present in the endoplasm.

Dobell¹ summarizes the present consensus of opinion regarding the pathogenicity of this organism. "At present . . . there seems to be no good evidence to support the hypotheses that *E. gingivalis* attacks the tissues, that it is the cause of pyorrhea, or that it is in any way pathogenic. The suggestion that the amoeba acts as a pathogenic agent by means of a 'symbiotic relation' with certain bacteria . . . seems equally unfounded. The organism appears rather to be a harmless commensal . . ."

Miller² reports that with nutrient gelatin as the medium he isolated 22 different bacterial species from 27 cases of pyorrhea alveolaris. He only names *Staphylococcus aureus* and *S. albus*, but one cannot feel sure from the text as to the frequency with which these were encountered. In another series of 12 cases, 20 different bacterial species were isolated. Here are included *S. aureus*, once; *S. albus*, once; *Streptococcus pyogenes*, once.

A year later Roughton³ expresses his view that ". . . any of the pyogenic bacteria may produce it (pyorrhea alveolaris) when the necessary predisposing causes have prepared the ground for it;" a view which is in the light of today's knowledge probably essentially true. He specifically mentions the pyogenic staphylococci and streptococci.

Kirk⁴ found pneumococci in the "pericemental abscesses" in teeth with living pulps, which abscesses are of periodontal, *i. e.*, pyorrhetic, origin.

Goadby⁵ tabulates the organisms seen on direct examination of smears of pus from the pockets as follows:

1. Cocci, generally in diplococci and massed around the epithelial cells in clumps.
2. Thick bacilli, generally jointed and often showing considerable irregularity in their staining characters.
3. Thick bacilli, with pointed ends and somewhat of the shape of a bean pod; they frequently show a division in the center and appear as two elongated triangles with the bases opposed.
4. Various fine bacilli, $0.5\ \mu$ and under in width, often exhibiting an irregular banded marking, especially well marked in the larger threads, which may be of great length.

¹ Amœbæ Living in Man, New York, 1919, pp. 98 *et seq.*

² Die Mikor-organismen der Mundhoehle, Leipzig, 2d ed., 1892.

³ Trans. Odont. Soc. Great Britain, 1893, 25, 71.

⁴ Dent. Cosmos, 1898, 40, 621.

⁵ Mycology of the Mouth, London, 1903.

5. Spirilla, spirochetes and comma-shaped bacilli, all showing marked motility in the hanging drop.

6. Varied yeast forms, sometimes with a partially developed mycelium.

7. Streptothrix threads, generally showing well-marked clubs.

8. Masses of bacilli associated with the threads, some jointed in chains, others free and often massed in clumps. Some of them exhibit polar staining.

He observes that although the cocci, as a rule, are present in only small numbers, nevertheless from pus containing all the forms summarized in the eight groups the cocci are the only ones which develop with any degree of certainty on artificial media. The ordinary pyogenic cocci are relatively infrequent. In 150 cases of marginal suppuration examined by Goadby exactly 10 per cent (15 cases) gave cultures of *Staphylococcus aureus* and *Staphylococcus albus*. The *Staphylococcus viscosus* (described by Goadby in peri-apical infections) was frequently encountered.

Goadby's interest continued in the flora of pyorrhea pockets, and found expression in studies published in the succeeding years. In 1905¹ his report was apparently limited to a consideration of the staphylococci. A broader study appeared a year later,² based on an experience of 300 cases. Both smears made directly with the pus and cultures were examined. He suggested that on a bacteriological basis three types of pyorrhea alveolaris might be distinguished:

1. One in which staphylococcal types predominated, such as *S. aureus*, *S. albus* and others; (2) a non-staphylococcal, mixed infection; (3) one caused by as yet undetermined organisms. In all of these three types two organisms were constantly encountered: (a) A bacillus, short and somewhat thick, three times as long as broad, growing at times into quite long threads, Gram-negative, staining feebly, motile, rendering milk acid and giving a positive Voges-Proskauer reaction, showing all things considered a close relationship to Jordan's *B. cloacæ*; (b) the *Staphylococcus viscosus*, to which reference has already been made.

In 1907 Goadby³ noted that in most diseased conditions about the mouth spirilla and spirochetes invariably appear in large numbers. These are conspicuous in smears made directly with the the pus. In cultures, from cases of pyorrhea alveolaris, cocci of one sort or another develop, and a streptococcus can always be

¹ Brit. Med. Jour., September 9, 1905, p. 562.

² Trans. Odont. Soc. Great Britain, 1906, 38, 145.

³ Lancet, March 9, 1907, p. 633.

isolated. Ninety cases (35 males and 55 females) were examined both by direct study of smears and by cultures. In 50 cases attention was limited to the superficial pus and in 40 cases the material was collected from deep in the pockets. The results of this investigation are tabulated below:

MICROSCOPICAL EXAMINATION OF PUS.

Morphological type.	Superficial pus.	Sockets deep.
Spirilla, spirochetes	37	19
Comma bacilli	32	11
Thick articulated threads	12	8
Thin articulated threads	5	7
Thick non-articulated threads	25	22
Thin non-articulated threads	24	26
Fine slender bacilli	6	27
Thick bacilli	11	7
Pointed bacilli	8	2
Streptobacilli	15	18
Short bacilli	13	21
Curved bacilli	9	12
Straight bacilli	5	2
Bean-shaped bacilli	1	3
Staphylococci	1	1
Diplococci	14	11
Cocci in masses	8	26
Streptococci	5	2
Isolated cocci	21	3
Sarcinæ	11	0

The results of the cultural examination of these same cases follow:

Morphological type.	Superficial pus.	Sockets deep.
Staphylococci	29	34
Cocci (brown colonies)	3	13
Cocci (<i>Staphylococcus viscosus</i>)	6	19
Streptococci	30	25
Sarcinæ	8	3
Motile bacilli	17	14
Streptobacilli	12	4
Short thick bacilli	6	12
Bacilli, forming threads	6	9
<i>B. maximus</i>	6	1
Yeast	2	3
Spirilla	2	3
"Bacilli"	12	14

Goadby¹ presented another report in this field in 1909. The microorganisms recognized in smears made directly with the pus include *Leptothrix racemosa*, *B. fusiformis*, diplococci, streptococci, streptobacilli, yeasts, a large number of diphtheroids and three varieties of spirochetes. Isolations were carried out on nutrient

¹ Lancet, December 25, 1909, p. 1875.

agar, under aërobic conditions. A large number of species were isolated and the opsonic test, using the patient's serum against the microorganisms recovered from his own pyorrhetic pockets, was employed to determine their respective pathogenic significance. In the light of this criterion the species of importance were: (1) Streptococci of the *longus* type, belonging to either *S. fecalis* or *S. anginosus* of Andrewes and Horder; (2) an occasional pneumococcus; (3) *Staphylococcus aureus* commonly; (4) *Staphylococcus citreus granulatus*;¹ (5) members of the *M. catarrhalis* group; (6) a diphtheroid.

Carmalt-Jones and Humphreys² were impressed by the streptococci in their cultures.

Simms³ remarked on the endless variety and the myriad numbers of bacteria to be seen on the direct examination of pus from pyorrhoea pockets. He notes cocci of all descriptions, bacilli, commas, spirals and many thread forms. He particularly stressed the fusiform bacilli and the spirochetes which were almost constantly present in enormous numbers. In regard to his cultures, the only observation of importance was the abundance of cocci, both staphylococci and streptococci.

Beebe⁴ regarded the isolated staphylococci and pneumococci as important. It is well to remember that some at least of the "pneumococci" may have been streptococci of the *viridans* or *alpha* type.

Eyre and Payne⁵ reported on the bacteriological examination of 33 cases. The direct microscopical study of the pus showed fusiform bacilli constantly present, numerous spiral rods, numerous filamentous forms, diphtheroids, many types of bacilli and numerous micrococci (staphylococci, streptococci, diplococci and tetracocci). Aërobic cultures on blood agar yielded various growths of which the following were regarded as significant: *Staphylococcus aureus*, 2; *M. catarrhalis*, 9; *M. catarrhalis* and *Streptococcus pyogenes longus*, 11; *Streptococcus pyogenes longus*, 7; pneumococcus, 4. Numbers represents the number of cases from which the organism or organisms were isolated.

Considerable attention has been given to the relation of bacteria to pyorrhoea alveolaris by Leary.⁶ He studied about 100 cases. The smears from the pus differed distinctly from the cultural findings. Fusiform bacilli and Vincent's spirochetes were constantly

¹ Freund: Inaug. Diss., Freiburg, 1898.

² Lancet, December 28, 1907, p. 1818.

³ Boston Med. and Surg. Jour., 1909, **161**, 613.

⁴ Proc. Roy. Soc. Med., Odont. Sect., 1909, **3**, 29.

⁵ Dent. Cosmos, 1910, **52**, 52.

⁶ Dent. Rec., 1908, **28**, 241.

present and the fusiform bacillus was regarded as an essential infecting agent in most cases of pyorrhea. The medium for the aerobic isolations was milk serum. Under these conditions perhaps the most constant finding was the pneumococcus. Short- and long-chained streptococci were frequently encountered. Staphylococci were rarely found alone but usually in association with pneumococcus or a streptococcus. It must not be forgotten that some bacteria identified as pneumococci in 1910 likely would, according to present criteria, fall in the group of the alpha streptococci. Blood agar and milk serum were the media used for anaërobic cultivations, which apparently gave nothing remarkable except a considerable variety of bacillary forms which did not grow well in aerobic cultures.

Streptococcus brevis and *M. catarrhalis* were found by Murrell¹ in pyorrhea pockets. MacWatters² found streptococci, *Staphylococcus aureus*, *M. catarrhalis*, pneumococci and a leptothrix. Sibley³ isolated streptococci, staphylococci and slender bacilli.

Georg Blessing⁴ isolated an organism closely resembling *Streptococcus pyogenes* with the greatest frequency. The other organisms found were *Staphylococcus aureus*, *S. flavus*, *S. albus*, pneumococcus and *Streptococcus longus*. The number of cases examined is not mentioned.

In 1912 Noguchi⁵ obtained in pure culture from a case of pyorrhea alveolaris a previously undescribed spirochetel form, which he named *Treponema mucosum*. This was not regarded as parasitic in the strict sense of the term, but it was observed to exert a certain pyogenous action when the tissues were so injured by foreign substances as to enable it to survive. Although Noguchi was naturally unwilling to pass upon its etiological significance, he was unquestionably of the opinion that the strong fetid odor in the discharge from pyorrhea alveolaris is due, at least in part, to the presence of the *Treponema mucosum* in the affected tissues.

A report of an examination of 115 cases was made by Medalia.⁶ The pus itself showed microscopically a great variety of bacteria, bacilli as well as cocci and spirochetes of all sizes and shapes. The bacilli which were very numerous were rarely found in cultures. All morphological types of the pyogenic cocci were frequent and

¹ Practitioner, 1910, **85**, 637.

² Proc. Roy. Soc. Med., Gen. Rep., 1910, **3**, 172.

³ Proc. Roy. Soc. Med., Odont. Sect., 1911, **4**, 71.

⁴ Zur Bakteriologie und antibakteriellen Therapie der Pyorrhœa alveolaris, Pfaff, Sammlung von Vorträgen a. d. Geb. d. Zahnheilk., Leipzig, 1911, H. 6.

⁵ Jour. Exper. Med., 1912; **16**, 194.

⁶ Boston Med. and Surg. Jour., 1912, **167**, 868.

at times a few short chains of streptococci were seen. Fourteen cases in the incipient stage on culture yielded pneumococci and staphylococci in 10 cases, pneumococci in 2 cases and staphylococci and streptococci in 1 case. The predominating organism isolated was a "pneumococcus in chains." The results of the cultural examination of 16 moderately advanced cases were: Pneumococci and staphylococci in 8 cases, pneumococci in 6 cases and staphylococci in 1 case. Again the predominating organism was the "pneumococcus in chains." Finally 85 far-advanced cases gave "pneumococcus in chains" in 18 cases, staphylococcus in 1 case, pneumococcus and staphylococcus in 49 cases, pneumococcus and streptococcus in 3 cases, pneumococcus and staphylococcus and streptococcus in 10 cases, pneumococcus and *M. catarrhalis* in 1 case and staphylococcus and *M. catarrhalis* in 1 case.

Medalia tried to incriminate the organisms which might be of particular significance in pyorrhea alveolaris. For this purpose he relied on the data furnished by the determination of the opsonic index and also by the results following the administration of auto-genous vaccins. In this way he decided that the coccal group was to be reckoned with rather than bacillary types. He concluded that the important infectious bacteria are the pus-producing forms, *i. e.*, pneumococci, staphylococci, streptococci and *M. catarrhalis*. The organism isolated with the greatest frequency (107 times out of 112 cases) was the "pneumococcus in chains." Although positive assertion is unwarranted, it is not improbable that this category included many types which today would be classed with the alpha streptococci. The difficulty of sharply distinguishing between the pneumococci and the streptococci must always be kept in mind in interpreting these reports.

Brown¹ reported on 42 cases. The isolations were made on blood agar under aërobic conditions. The organisms identified, together with the number of cases in which they were found, follows: *Staphylococcus aureus*, 6; *Staphylococcus albus*, 9; *S. sarcina*, 3; *M. tetragenus*, 9; Gram-negative cocci, 49; Gram-positive bacilli, 17; *B. influenzae* (group), 19; streptococcus (non-hemolytic), 28; streptococcus (hemolytic), 30; *Streptococcus viridans*, 23; pneumococcus, 27; diplococci, 13; diphtheroids, 22; yeast, 1; leptothrix, 3; bacillus, 1.

Some of the work of Brown was familiar to Head.² This latter author reported on 40 cases. In about 25 per cent *Streptococcus*

¹ New York Med. Jour., 1913, 98, 1201.

² Jour. Am. Med. Assn., 1913, 61, 2232.

viridans was present. The other organisms isolated included other streptococci, *Staphylococcus aureus*, *S. albus*, *B. influenzae*, pneumococci, *M. catarrhalis*, diphtheroids, Friedländer's bacillus and an occasional unidentified form. A few years later Head and Roos¹ published their experiences in over 350 cases of pyorrhea alveolaris. The cultivations were conducted aerobically. Streptococci were present in 95 per cent of the cases. About 80 per cent of the cases yielded representatives of the *M. catarrhalis* group and of the hemoglobinophilic (*B. influenzae*), group. Pneumococci, staphylococci, diphtheroids, Friedländer's bacillus, *M. tetragenus* and a few unidentified species were also isolated.

The extensive experience of Hartzell and Henrici in this field may be justly summed up in the statement that streptococci are constantly present in pyorrhea alveolaris,² and constitute the group of greatest importance.

Although spirochetes had been observed in pyorrhetic pockets by the earliest investigators in this field, renewed interest in this group followed the introduction of arsphenamin and neoarsphenamin. Gerber incidentally mentioned that these microorganisms were demonstrable in many oral inflammations, including clinical pyorrhea alveolaris. Kolle³ examined microscopically the pus from 90 cases. He noted a luxuriant flora, Gram-positive and Gram-negative cocci, bacilli, thread forms and spirochetes. He was convinced that a peculiar spirochete, previously unrecognized, was the dominant type. The microorganism in question morphologically greatly resembled *S. obermeieri*. It was 10 μ to 12 μ long; the number of curves averaged 5 but varied from 4 to 7 ends for the most part pointed; staining faintly with Giemsa solution but well with the ordinary gentian violet. Examined by the dark-field method, it showed flexion, rotation and locomotion. To it the name of *Spirochæta pyorrhæica* was assigned. In most cases examined this microorganism appeared to be present in pure culture, often only associated with very few bacteria. Among such bacteria those of the fusiform type were most conspicuous. Although other spirochetes were present, Kolle regarded the *S. pyorrhæica* as playing the dominant if not the only role in pyorrhea alveolaris.

This thesis was supported by Beyer,⁴ but was seriously called into question by Seidel.⁵

¹ Pacific Dent. Gaz., 1919, **27**, 713.

² Jour. Am. Med. Assn., 1915, **64**, 1055.

³ Med. Klin., 1917, **13**, 59.

⁴ Med. Klin., 1917, No. 5; *ibid.*, 1918, Nos. 3 and 4; Zahnärztl. Rundsch., 1918, No. 28; Alveolar-pyorrhoe als lokale Spirochætose der Mundhöhle, Berl. Verlag., 1919.

⁵ Deutsch. Zahnk., 1919, H. **41**.

The following microorganisms were isolated by Lescohier¹ from 70 cases of pyorrhea alveolaris. The number indicates the number of cases in which each appeared: Streptococci, 19; pneumococci, 5; *B. necrodentalis*, *M. catarrhalis*, 23; *B. septus*, 15; *Staphylococcus aureus*, 12; *M. citreus granulosis*, 6; saccharomyces, 5.

Drew and Griffin² systematically examined some 300 cases. They were greatly impressed with the immense numbers of spirochetes to be seen in smears from typical cases of pyorrhea alveolaris, and express the belief that these microorganisms play the chief part in the pathogenesis of this condition. They recognized at least six species of spirochetes, viz: *Treponema macrodentium*, *T. microdentium*, *T. mucosum*, *Spirochæta buccalis*, *S. vincenti* and *S. refringens*. They also found streptococci in every case, and fusiform bacilli and vibrios were often extremely numerous. Among the isolated species were *M. catarrhalis*, *Staphylococcus albus*, *S. aureus*, *M. tetragenus*, large numbers of leptothrices and some unidentified bacteria.

Mendel³ mentions three anaërobes isolated from pyorrhetic pockets: (a) *Staphylococcus parvulus* Veillon, found in all of the 12 cases examined; (b) a small "Vibron du Type *Repaci* B," found in 7 of 12 cases; (c) an unidentified Gram-negative sporogenous bacillus, found in 3 of 12 cases. Mendel, by intragingival injection of pure cultures into rabbits tried to reproduce pyorrhea alveolaris. In this respect *Staphylococcus parvulus* gave the best results. But it is noted that the inoculated organism rapidly disappeared from the pus and was supplanted by a polymicrobial flora. In this pus neither amoeba nor "spirilles" (spirochetes?) were seen.

Kritchevsky and Séguin⁴ studied the distribution of spirochetes in 244 subjects. In three-fourths of the cases of pyorrhea alveolaris spirochetes were abundant. If the patient had not previously undergone any specific treatment there is in almost all cases (24 as against 2) an abundance of spirochetes. The cases of pyorrhea in which there are only a few or even none at all of these organisms (11) are particularly to be seen in individuals already treated. When pyorrhea alveolaris is in its initial stages spirochetes are found in one-half the cases (31 out of 62). When the mouth is healthy the spirochetes are rare or absent in about three-fourths of the cases (101 out of 139).

In one-half of 110 cases never having had specific treatment

¹ Jour. Am. Med. Assn., February 10, 1917.

² Jour. Roy. Microsc. Soc., London, April, 1917.

³ Pyorrhée alvéolaire expérimentale, Compt. rend. Soc. de biol., 1917, 80, 962.

⁴ Presse méd., May 13, 1918; translated in the Dent. Cosmos, 1918, 60, 781.

spirochetes are abundant in the gingival exudate. Of 143 subjects already treated by specific medicaments (mercury, "606" or "914"), spirochetes are found in only one-third of the cases.

Cavalié and Mandoul¹ described under the name of *Spirochæta perforans* (n. sp.) a form which they constantly found in pyorrhetic pockets. It is 10 μ to 12 μ long, with waves about 2 μ in diameter. The ends are blunt and no flagella were demonstrated. These same authors later² reported that in all but 2 of 27 cases of pyorrhea alveolaris spirochetes were microscopically found before treatment.

Turner and Drew³ found staphylococci, streptococci of the type of Gordon's *S. pyogenes* and diphtheroids. In one instance a diphtheroid appeared on culture which showed many affinities to *Bacillus septus* (*B. coryzæ contagiosa*). This diphtheroid showed marked complement fixation with the patient's serum. On another occasion there was observed a heavy infection with a sporogenous bacillus.

Seitz⁴ found staphylococci, *Streptococcus brevis*, pneumococci, *Streptococcus lacticus*, anaërobic and facultative rods, spirochetes and fusiform bacilli. He regarded the bacterial phase of pyorrhea alveolaris as a mixed infection.

Thirty-eight cases were examined culturally by Coxon.⁵ Streptococci were isolated from 37 cases; staphylococci from 10; *M. catarrhalis* from 21; Gram-negative micrococci from 11; Gram-positive micrococci from 13; coliform bacilli from 5; fusiform bacilli from 1 case.

Perrin⁶ found bacteria present in 100 per cent of cases, but these frequently are without pathogenic significance. Pneumococci were found in 75 per cent of cases; *M. catarrhalis* in 60 per cent; fusiform bacilli in 38 per cent; diphtheroids in 22 per cent; streptococci in 20 per cent and staphylococci in 18 per cent. Trichobacteria, including *Leptothrix buccalis maxima*, *L. racemosa*, *L. innominata* and the *B. buccalis maximus* were present in 60 per cent. Spirochetes were present in 100 per cent of the cases; the genus *spironema* in 100 per cent, *treponema* in 100 per cent and *leptospira* in 35 per cent. Perrin regards pyorrhea alveolaris as essentially a spirochetosis frequently complicated by a polymicrobial infection.

Salivary Calculus.—Although the evidence available seems to contradict the view that the formation of calcareous deposits about the

¹ Compt. rend. Soc. de biol., 1921, **85**, 1068. ² Presse dent., 1922, **24**, 308.

³ Proc. Roy. Soc. Med., Odont. Sect., 1919, **12**, 104.

⁴ Deutsch. Monatschr. f. Zahnk., 1921, **39**, 34.

⁵ Brit. Dent. Jour., 1922, **43**, 106.

⁶ Rev. Mex. de biol., 1922, **2**, 171.

necks of the teeth is a primary or essential factor in periodontoclasia, these deposits are so frequently found that they cannot be ignored in this discussion. The possibility that bacterial action may play a part in the origin of salivary calculus has been suggested by Bulleid¹ and by Hall and Westbay.² Precipitates occurred in nutrient broth, to which CaCl_2 had been added, when inoculated with pure cultures of *Leptothrix buccalis* which had been isolated from the human mouth. In one case where the calcium content originally had been 8 mg. per 100 cc after six days it was 1.4 mg. per 100. The pH of these tests was about 7.6 and 6. Partial detachment of the periodontal gingivæ of cats and introduction into such pockets of pure cultures of human strains of *L. buccalis* was followed by deposits of calculus. The following table adapted from Hall and Westbay shows that with increasing alkalinity the calcium content of saliva decreases.

REACTION CHANGES IN SALIVA IN RELATION TO PRECIPITATION
OF MINERAL SALTS.

Time.	pH.	Calcium, mg.
At once	7.6	4.6
Three hours	7.8	4.4
Nine hours	8.0	3.8
Twenty-one hours	8.0	2.9
Two days	8.4	0.3
Three days	8.6	Trace
Four days	8.6	Trace

The changes in the reaction of the saliva are, of course, in part due directly to bacterial activities. The alkalization of saliva requires time, a condition, of course, met by the stagnation which has been emphasized by Prinz.³ The hypothesis of this author regards the formation of salivary calculus within the oral cavity as primarily a physico-chemical process which at times may be secondarily enhanced by chemical reactions. The observations of Bulleid and of Hall and Westbay do not seem to be incompatible with this view, but may contribute to its amplification.

Summary.—The above review of various bacteriological examinations makes the infectious phase of periodontal disease seem more complicated than probably it really is. In the light of present knowledge no single species of microorganism is invariably required for the pyorrhea and usually, if not always, in any particular case

¹ Guy's Hosp. Repts., 1924, **74**, 444.

² Dent. Cosmos, 1925, **67**, 115.

³ Dent. Cosmos, 1921, **63**, 369, 503.

two or more types of known pyogenic powers can be isolated or demonstrated. In other words, the pyorrhea alveolaris is probably the result of a mixed infection and is a condition without specific etiology.

The literature summarized roughly falls into two categories: (1) That laying the major stress on the ordinary pyogenic cocci, and (2) that emphasizing the importance of the spirochetal forms.

The pyogenic cocci involved include *Staphylococcus aureus*, *S. albus*, *S. citreus* and other less definitely known staphylococci, *M. catarrhalis*, *M. tetragenus*, pneumococci and streptococci. These organisms are always conspicuous in cultures made under aërobic conditions. Their relationship to the flow of pus is at least suggested by the constancy of their presence, by the demonstration of corresponding antibodies, *e. g.*, opsonins, in the patient's blood, and by the clinical improvement sometimes following the administration of autogenous vaccins of which they are components. There has been a tendency lately to consider the part played by the short-chained streptococci as one of peculiar significances. This finds strong expression in Glynn's excellent résumé.¹ ". . . there is no doubt that the streptococci are the chief pathogenic aërobic organisms in periodontal infections." And later: "But once the resistance is lowered and perhaps inflammation established *streptococci, particularly the non-hemolytic oral streptococci, are the chief bacteria, which if they do not cause the gingivitis or pyorrhea at least perpetuate and often aggravate it.*"

In contrast to this view is the view of those who look upon the spirochetes as the principal if not the sole microorganisms of significance. These microbes are present at least as constantly as the pyogenic cocci. In untreated cases they often numerically completely overshadow all other forms, and the clinical improvement reported following the administration of reputed spirocheticides is associated with a more or less striking diminution in the numbers of the spirochetes. Finally, Kritchevsky and Séguin² have tried to demonstrate that these microorganisms are potentially pathogenic in laboratory animals and that fusiform bacilli and spirochetes are conspicuously present in the affected human tissues.

It should be noted that these authors propose a conception which in a way is a compromise. The initial infection of the tissues is attributed to the joint action of spirochetes and fusiform bacilli.

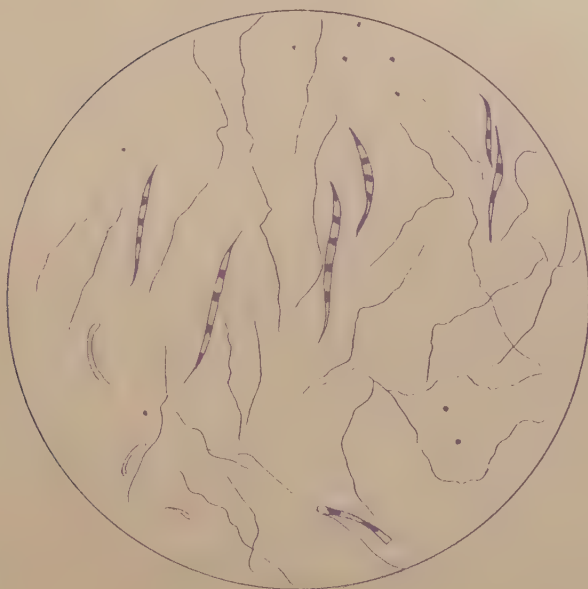
¹ Diseases of the Periodontal Tissues due to Infection, etc., Dent. Board United Kingdom, London, 1923, p. 87.

² Dent. Cosmos, 1921, **63**, 889; and *ibid.*, 1924, **66**, 622.

At this stage pus is not present. This is the so-called dry "pyorrhea." Later when the damage done permits the retention of the cocci these organisms exert their pyogenic action, contribute its characteristic feature to the disease and accelerate its progress.

In conclusion it is very likely that the pyogenic cocci have a share and the same must be said for the spirochetes and fusiform bacilli. The sequence in which these forms act and their relative importance, as suggested by Kritchewsky and Séguin, are purely hypothetical. We must admit that at the present time it is impossible to evaluate accurately the roles of the several microorganisms concerned.

PLATE IV



Smear from Vincent's Stomatitis.

Oil immersion lens. Anilin-oil-gentian violet stain. Fusiform bacilli
and the spirochetes of Vincent

CHAPTER XXIV.

VINCENT'S INFECTION.

THE condition to be considered at this time goes under a great variety of names, *e. g.*, "trench mouth," Vincent's gingivitis or stomatitis and ulcero-membranous stomatitis. The microorganisms, whose presence is characteristic of these infections, are the fusiform bacillus and the spirochete of Vincent (Plate IV). There still exists some doubt as to the pathogenicity of these species, but they are at least of diagnostic importance. It is certain that at times predisposing factors share a large part of the responsibility. Besides dominating the field in ulcero-membranous stomatitis, fusiform bacilli and spirochetes, or the fuso-spirillary symbiosis, as it is often called, also appear in a great variety of oral and extra-oral conditions, *e. g.*, mercurial stomatitis, the stomatitis of scurvy, noma, a conjunctivitis, an otitis media, a tonsillitis, a meningitis, a balanitis, a bronchitis (fetid and hemorrhagic), a form of pulmonary gangrene, as secondary invaders in the cavities of pulmonary tuberculosis, and a rare type of dysentery. A cutaneous infection on the finger followed the bite of an insane man suffering from Vincent's stomatitis; involvement of the external auditory meatus is known.

General invasions have occurred and fatal results are reported by King,¹ Husik,² and Mutermilch and Séguin.³

The term Vincent's *angina* is applicable only to the tonsillitis associated with Vincent's microorganisms. It is incorrect to speak of a Vincent's stomatitis as an angina, for this term implies a difficulty in swallowing.

The characterizations of the organisms, which follow, are based on those in Bergey's *Determinative Bacteriology* (first edition).

Fusiformis Dentium.—Rods with pointed ends, fusiform, *i. e.*, spindle shaped, $1.5\ \mu$ to $4\ \mu$ by $3\ \mu$ to $10\ \mu$, showing from 2 to 6 deeply staining granules, often slightly curved, non-motile, no endospores, results with Gram's stain variable,⁴ anaërobic, requiring serum or similar protein in media for isolation. It is known that

¹ California State Jour. Med., 1918, July.

² Husik: Ann. Otol., Rhinol. and Laryngol., 1922, **31**, 1039.

³ Compt. rend. Soc. de biol., 1923, **88**, 28.

⁴ Gifford: Jour. Bacteriol., 1920, **5**, 365.

there are several types or species of fusiform bacilli, although it is conventional to group them all under the designation of *B. fusiformis* or (as a synonymous term) *Fusiformis dentium*. They may occur independently of the spirochetes, *e. g.*, Veillon and Zuber¹ reported these organisms in pure culture in an inflamed appendix, and Worster-Drought² found them in the spinal fluid of a case of meningitis.³

The spirochete of Vincent should preferably be termed *Borrelia vincenti*. It is a very delicate, flexible, motile spiral, about 0.3 μ by 12 μ to 25 μ long. The number of waves or curves in its body is usually about 4 to 5. These waves are not uniform in either height or amplitude. Strict anaërobiosis and the presence of serum or some similar protein in the medium are necessary for its isolation.

It has been claimed by Tunnicliff⁴ that the fusiform bacillus and spirochete of Vincent are merely different developmental forms of one and the same organism. At the present time the consensus of opinion probably is that this is an erroneous conception.⁵

Phillips and Barry⁶ have found Vincent's organisms in dogs, usually in relatively small numbers but occasionally in great numbers associated with gingival disease. Klarenbeed⁷ found fusiform bacilli quite constantly in the oral cavities of mammals. In the ruminants examined and in the rabbit they were not associated with spirochetes. In the normal as well as in the diseased oral cavity of the dog and cat, both organisms are encountered together. They have also been seen in the horse. I have seen smears from the gingivæ of a cat, which could not have been distinguished from smears coming from indubitable cases of Vincent's stomatitis in man. In view of the high infectiousness of Vincent's organisms, as appeared in the World War, these facts clearly suggest the possibility that cats and dogs may serve as reservoirs for these organisms and may play a part in their dissemination particularly among children.

The symptomatology and laboratory findings of ulcero-membranous stomatitis have been very satisfactorily given by Barker and Miller.⁸ Constitutional disturbances are, as a rule, insignificant; the patient is not very ill. Fever is usually absent and rarely

¹ Arch. de méd. expr. et d'anat. path., 1898, 10, 517.

² Brit. Dent. Jour., June 15, 1918.

³ See also Dick: Jour. Infect. Dis., 1913, 12, 2.

⁴ Jour. Infect. Dis., 1906, 3, 148; *ibid.*, 1911, 8, 316; *ibid.*, 1923, 33, 147.

⁵ Krumweide and Pratt: Jour. Infect. Dis., 1913, 13, 438.

⁶ Jour. Infect. Dis., 1920, 27, 136.

⁷ Tijdschr. v. Diergeneesk., Utrecht, 1922, 49, 495.

⁸ Jour. Am. Med. Assn., September 7, 1918.

over 100° to 101° F. The breath is heavy and offensive. Moderate enlargement of the cervical and submaxillary lymph nodes occurs; they are tender but never suppurate. The gums are swollen, spongy and bleed easily, suggesting scurvy. In more advanced cases salivation is marked, the teeth loosen and mastication may be so painful as to preclude eating. Subjectively the patient is fatigued and often mentally depressed. Cultures taken from the circulating blood to determine if a bacteriemia exists have been uniformly negative.

The Wassermann reaction in all cases of Vincent's infection is negative in non-syphilitic patients. The contrary view that the Wassermann was positive was rather widely held at one time but it is absolutely unsupported by fact. Taylor and McKinstry¹ performed this test on 55 individuals exhibiting typical Vincent's angina. Negative results were obtained in all but 2, who admitted syphilitic infection. The presence of a positive reaction does not exclude Vincent's disease, and experience has shown that patients with a syphilitic history, undergoing mercurial treatment, are especially prone to this type of stomatitis.

Tarnow² has given considerable attention to the examination of the blood in Vincent's angina. He found the blood platelets (8 cases) constantly normal in number; the coagulation time of the venous blood (5 cases) normal; the hemoglobin (2 cases) showed inconsequential variations. The number of erythrocytes (2 cases) was normal; this being corroboratory of the finding of Barker and Miller, who noted that anemia does not develop in the majority of instances. These latter authors also observed that the leukocytes are rarely increased over 10,000, while Tarnow in early stages of the infection found in 8 cases a leukocytosis of from 8000 to 14,000. The following table gives the differential leukocyte count for these 8 cases.

Case.	Polymorphonuclear neutrophils.	Lymphocytes.	Monocytes.	Eosinophils.
1	49.66	29.66	19.33	1.33
2	55.00	32.00	12.66	0.33
3	55.00	30.33	13.67	1.00
4	53.66	33.00	11.33	2.00
5	64.70	21.30	13.00	1.00
6	57.33	37.00	5.66	0.00
7	27.00	45.00	27.00	1.00
8	60.00	30.50	6.00	3.50

From inspection of this table it becomes apparent that the percentage of mononuclears (lymphocytes and monocytes) is raised at

¹ Brit. Med. Jour., January 19, 1918, No. 2977.

² Med. Klin., 1921, 17, 1024.

the expense of the polymorphonuclear neutrophils. Goadby¹ agrees with this. "In the absence of secondary infections the differential blood count shows polymorphonuclear leukopenia with relative lymphocytosis." Hemoglobin is low but the number of red cells is normal in the early stages of the disease. A hyperleukocytosis indicates secondary infection with some other organisms than Vincent's. The absence of a neutrophilic hyperleukocytosis is suggested by the fact that in smears made directly from the lesions the blood cells of an inflammatory exudate are conspicuous by their absence in uncomplicated cases of Vincent's infection.

When the neutrophils are grouped according to Arneth's classification there appears a "displacement to the left" indicating that the reaction is not only local in its influence but also involves an increase in the activity of the bone-marrow.

Peter² reports four differential blood counts in the same case of Vincent's angina.

	Neutro- phils.	Eosino- phils.	Lympho- cytes.	Mono- nuclears.	Mast cells.
Oct. 16 . .	66	8	22.0	3	
17 . .	67	11	17.5	4	0.5
23 . .	51	8	32.0	6	3.0
Nov. 18 . .	65	3	30.0	2	

This shows conspicuous differences from the findings of Tarnow. The neutrophils are not diminished; the lymphocytes show less increase; the mononuclears are much lower, while there is a decided eosinophilia. Peter believed he had excluded the other conditions which might explain the eosinophilia. There were also many eosinophils in the smears made directly from the tonsillar lesion.

Tarnow also pointed out a criterion between diphtheria and Vincent's angina, which are often clinically confusable. In diphtheria the percentage of neutrophils rises to the higher values, *e. g.*, in 5 consecutive cases, 80.33, 74.33, 80, 74.66 and 59, while in Vincent's infection this percentage is rather at the lower margin of the normal.

Barker and Miller and also Goadby report that a transient albuminuria is common in the more severe cases.

The histology of the tissues locally involved has been described by several. Tunnicliff³ had opportunity to study sections of a tonsil that had been removed during life, *i. e.*, the possibility of

¹ Diseases of the Gums and Oral Mucous Membranes, Oxford Med. Pub., 1923, p. 41.

² Deutsch. med. Wehnschr., 1923, 49, 279.

³ Jour. Infect. Dis., 1919, 25, 132.

postmortem changes was eliminated. The surface exudate contained cocci, a variety of bacilli and a few spirochetes. Under the exudate the external epithelium is necrotic and contains only an occasional coccus, fusiform bacillus and spirochete. The layer between this necrotic tissue and the living tissue contains a few leukocytes, a moderate amount of fibrin, and enormous numbers of fusiform bacilli and spirochetes. The former organisms are often packed together in palisade arrangement. The necrotic lymph follicles are replaced by a mass of fusiform bacilli and spirochetes, the latter being almost in pure culture at the center of the follicle with the bacilli radiating out from them. The fusiform bacilli and spirochetes are seen invading the living tissues, the latter generally just ahead or considerably in advance of the fusiform bacilli. Sections stained to bring out the commoner types of bacteria (methylene blue and Gram-Weigert) show cocci only in the exudate and in the external layer of the tonsil. They are not associated with the fusiform bacilli and spirochetes near or in the living tissues. Kritchewsky and Séguin¹ have furnished corroboratory evidence of this nature relative to the pathogenicity of these microorganisms. In discussing this problem, Barker and Miller call attention to three significant facts:

1. In all the lesions under consideration these organisms are present, as a rule in enormous numbers and often virtually in pure culture. The severer the infection the greater the number.
2. There is positive evidence both as to the infectiousness and contagiousness in Vincent's disease.
3. Healing of the lesions goes on parallel with the disappearance of the organisms. When complete, no organisms remain.

Before concluding mention should be made of the relation of the condition of the mouth to this infection. Oral sepsis, periodontal gingivitis or pyorrhea alveolaris are favorable for the development of ulcerative stomatitis. Furthermore, once the disease is established thorough hygiene of the mouth is necessary for cure. Sole reliance should not be placed on topical applications. It is requisite that septic crowns and bridges and decayed roots be radically removed.

¹ Dent. Cosmos, 1921, **63**, 888; *ibid.*, 1924, **66**, 622.

CHAPTER XXV.

GONOCOCCAL INFECTION.

THE venereal disease, second in importance to syphilis, is gonorrhea. It is caused by a bacterium, known commonly as the gonococcus and also as *Neisseria gonorrhæa*. It is a Gram-negative principally intracellular diplococcus, biscuit-shaped or coffee-bean-shaped (Fig. 85). Its position within the polymorphonuclear neutrophils is a result of the phagocytic function of these cells. As many as 122 gonococci have been found within the cytoplasm of a single cell, although the average number was 32.¹

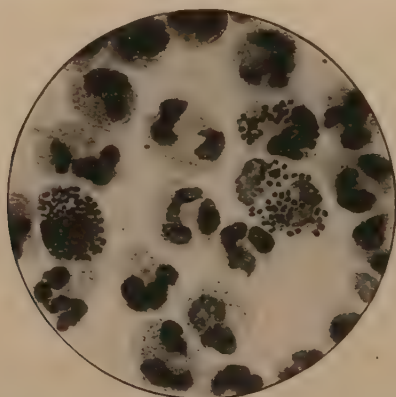


FIG. 85.—Gonococcus within pus cells, fuchsin stain. $\times 1000$ diameters. (Fränkel and Pfeffer.)

Besides the genital and conjunctival forms of gonorrhea, with arthritic and cardiac complications, there has been described as a rarity, gonococcal stomatitis. This occurs in two forms, in infants and in adults. In the former it is usually a disease acquired at birth; in the latter it is ordinarily contracted as an auto-infection secondary to the genital localization or during perverted coitus. The incubation period, which in genital gonorrhea is about six to seven days, is reduced often to merely one or two days. A rawness or a dryness of the mouth is first noticed. Small herpetic ulcers may

¹ Oelze-Rheimboldt, M.: Centralbl. f. Bakteriol., I. Abt., Orig., 1921, **81**, 28.

appear upon the lips. The entire oral mucosa becomes bright red, intensely inflamed and highly sensitive. A very offensive exudate, whitish and mixed with blood, is present. Epithelial desquamation occurs and small areas may be noted covered with an easily detachable false membrane. The gums are much swollen and retracted from the teeth. The appearance resembles a severe case of mercurial stomatitis (Vincent's stomatitis). Pus is discharged from around the necks of the teeth, which eventually are exfoliated unless the condition is arrested.

The tongue is swollen and very sensitive. Its surface is glazed and marred with small superficial ulcers, secreting a thick yellowish pus. The breath is very offensive. Salivation may be slight or abundant, viscous and ropy. The submaxillary nodes and lymphatics may be affected.

The systemic reaction may be marked, manifesting itself in chills, fever, headache, lassitude, and malaise. Definite diagnosis cannot be made without the recognition of gonococci in stained smears from the lesions.

A gonococcal parotitis has been described as a postoperative complication.¹

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CHAPTER XXVI.

TUBERCULOSIS AND SYPHILIS.

TUBERCULOSIS.

TUBERCULOSIS is one of the commonest and most serious diseases affecting man. In the registration area of the United States for 1919, there were reported in all 106,985 deaths ascribed to tuberculosis. This represents 9.8 per cent of all deaths for that period and is only exceeded by "organic diseases of the heart," responsible for 10.2 per cent of all deaths.

It is impossible to estimate with any degree of accuracy the number of clinically recognized cases of tuberculosis, corresponding to this mortality; but it is known to be extremely great. Tuberculin tests and the evidence of autopsies indicate that a very large proportion of the entire population of any civilized country is or has been at one time or other infected with tubercle bacilli. It is true that the vast majority of these people are not aware of the existence of such infection. Autopsies reveal case after case in which at the examination of the lungs the knife meets one or more hard, gritty calcified nodules at the hilum or in the substance of the lungs or in the mediastinum. These represent healed or quiescent tubercular lesions. The apices of the lungs also frequently show the peculiar scarrings or puckerings indicative of the same condition. Most of these cases give no history of diagnosed tuberculosis. At most there is sometimes a suggestion, such as a period of a few weeks or months when the patient lost weight or felt poorly, regaining normality often without medical attendance.

These facts indicate that the human species in general is very susceptible to tubercular infection and that also the defensive response is in most instances adequate to protect the patient.

Tuberculosis of the lungs occurs far more frequently than all other forms combined. For instance of the 106,985 deaths for 1919, 94,772 were due to pulmonary localization. Tubercular meningitis accounted for 5175 while all other forms of tuberculosis combined caused only 7038 deaths. The recollection of these data will help us to be sanely orientated when later we center our attention upon the oral aspects of this disease.

The exciting microbic cause of tuberculosis is the tubercle bacillus (*Mycobacterium tuberculosis*). From the stand-point of human medicine there are two well-recognized types which are not easily differentiated, the human and the bovine type. The latter as the name indicates is the one most frequently encountered in tuberculosis of the domestic cattle. It is also infectious for man. The number of deaths caused by it in individuals of all ages constitutes about 6 per cent of all deaths due to tuberculosis.¹ Of the deaths occurring from tuberculosis in children under five years of age, the bovine type seems to be the agent in about 33 per cent. The virulence of the human type for domestic cattle is not high. Cattle present a greater resistance to infection with the human type than human beings offer to the bovine type.

The demonstration of delicate, beaded, rod-shaped, acid-fast bacteria in material from a lesion, suspected clinically of being tubercular is usually considered as confirming the diagnosis. However, failure to find such bacteria on microscopic examination does not mean the absence of tubercular infection. In event of such failure any inference as to presence or absence of any particular infection is unwarranted. A negative result must be regarded as entirely noncommittal. (Plate I, Fig. 2.)

Where microscopic demonstration of the tubercle bacillus fails, recourse is made to animal inoculation. The guinea-pig is most susceptible.

List of Acid-fast Bacteria.—*B. tuberculosis* (all strains; human, bovine, avian, reptilian, amphibian and piscian).

B. lepræ.

Certain pathogenic strains of actinomyces or streptothrix.

B. smegmæ (non-pathogenic).

Bacillus of verruga peruana (an Andean disease).

Acid-fast bacilli other than the above have been found in milk, butter, manure, grass, and air; *e. g.*, Moeller's "hay bacillus," his "timothy-hay bacillus," and the "butter bacillus" of Petri and Rabinowitsch. Some of these acid-fast microorganisms are pathogenic at times for man; *e. g.*, in pulmonary gangrene, in various pulmonary diseases and in diseases of the alimentary canal.² Abbott and Gildersleeve³ give a summary of the literature to 1902 of the acid-fast bacteria other than the tubercle bacilli.

The Acid-fast Property—There is still much disagreement as to whether the acid-fastness of tubercle bacilli depends upon waxes,

¹ Cobbett: Causes of Tuberculosis, Cambridge Univ. Press, 1917.

² Besson: Practical Bacteriology, Microbiology and Serum Therapy, 1913, p. 345.

³ Univ. Pennsylvania Med. Bull., June, 1902, p. 3.

alcohols, fatty acids, or lipid-protein compounds. At least one factor in the acid-fastness is the integrity of the bacillary envelope.¹ The substance responsible for the acid-fast property is insoluble in alcohol or ether. Treatment of the bacilli with warm xylol extracts a waxy substance, which is itself acid-fast, while the bacilli have lost that property.²

In a very elaborate chemical analysis of large quantities of dried tubercle bacillus (human and bovine) Goris³ found that the fatty, waxy or lipoidal substances of the bacillus comprised:

1. Hyalinol.

2. A waxy mixture containing two alcohols: Mykol, m. p. 65° C. and another alcohol considered to be lecithin.

3. Among the fats extracted, the glycerids of oleic, stearic, palmitic, arachidic, caproic and butyric acids were recognized, and also myristic and "isocetic" acid.

The resistance of the tubercle bacillus to decolorization by acids after being stained was found to be due to certain lipoidal constituents, notably the free fatty acids and the waxes.

Tuberculosis and Dentistry.—Tuberculosis, and by this is meant almost exclusively pulmonary tuberculosis, is thought by some to influence or to determine certain oral conditions. Brown⁴ notes that the gums in advanced cases, owing to poor nutrition, are often spongy and soft. Pyorrhea alveolaris is not infrequent. A bluish-red line on the gums, first described by Fredericq in 1851, is not peculiar to pulmonary tuberculosis, but occurs in many wasting diseases and in some healthy persons. Bleeding of the gums is very common. Caries of the teeth is frequent and is of great importance on account of the need for thorough mastication. The condition of the tongue varies directly with the condition of the patient. In incipient cases with good digestion it is clean, moist and normal, as also in many advanced cases. When gastric complications occur or when the patient sleeps with an open mouth, the tongue is usually covered with a white fur, which may become dark from food or medicine. In advanced stages sordes or a deeply fissured tongue may be present.

Siffre⁵ denies any peculiar influence of tuberculosis in dental caries. Roddy, Funk and Kramer⁶ concluded that pulmonary tuberculosis predisposes to pyorrhea alveolaris or the condition

¹ Wells, H. G.: Chemical Pathology, 4th ed., 1920, p. 106.

² Besson: Practical Bacteriology, Microbiology, Serum Therapy, 1913, p. 306.

³ Ann. de l'Inst. Pasteur, Paris, 1920, **34**, 497.

⁴ Osler's Modern Medicine, 1907, **9**, 263.

⁶ New York Med. Jour., 1916, **104**, 433.

⁵ Progrès méd., 1922, **37**, 580.

favoring the development of pulmonary tuberculosis also favors the development of "pyorrhea." Of 1776 patients with pulmonary tuberculosis 80 per cent presented distinct clinical signs of "pyorrhea" while the highest incidence among similar but non-tuberculous groups was 15 per cent. The pigmentation of the oral mucosa in Addison's disease (tuberculosis of the adrenals) has long been known.

Tubercular lesions of the oral cavity and adjacent parts are very rare. Only 4 or 5 cases were found by Heller¹ among 8000 patients of a laryngological clinic. Morrow and Miller² found in 1444 tuberculous patients about 1 per cent who showed tuberculosis of the tongue as a secondary involvement. It may affect the tongue, gingivæ, mucosa of lips, cheeks, palate or uvula, the tonsils and faucial pillars, the mandible and the maxillæ, the maxillary sinus, the salivary glands, and the cervical lymph nodes. Histologically recognizable lesions have been found in the dental pulp.³ When oral tuberculosis does occur it is almost always secondary to some other localization, usually in the lungs. Lesions primary in or about the mouth are of the greatest rarity—so much so that when an oral tubercular lesion is recognized, every effort should be made to discover other (and more likely primary) lesions. Sometimes the diagnosis of oral tuberculosis is the first intimation that the patient has any pulmonary involvement.⁴ Although it is difficult to eliminate the possibility that the bacilli have reached the oral tissues by blood or lymph channels, it is more likely that the bacilli contained in infectious sputum are simply implanted upon already more or less diseased, although not tubercular, areas where the mucosal epithelium is abraded. The microorganisms concerned have been repeatedly found in the mouths of patients with pulmonary tuberculosis and even, though more rarely, in the mouths of the apparently non-tubercular.

Cook reports⁵ that he has found tubercle bacilli in 11 out of 200 individuals. His method of study is very incompletely described, but apparently these findings were based upon the direct microscopic examination of smears from the surfaces of teeth, cavities of carious teeth, pulp-chambers of pulpless teeth, and saliva, stained for acid-fast organisms. Consequently at most his findings indicate the presence of an acid-fast bacterium; and not by any means

¹ Zilz: *Tuberkulose der Mundhoehle* Wien., 1912, p. 80.

² *Jour. Am. Med. Assn.*, 1924, **83**, 1483.

³ Ivy and Appleton *Jour. Am. Med. Assn.*, 1923, **81**, 1483.

⁴ *Dent. Rev.*, 1899, **13**, 97.

⁵ Zily: 1912.

necessarily the true, pathogenic bacillus of Koch. In 5 of the 11 positive cases, there was clinical evidence of tubercular infection. Hoppe (Tellier) found tubercle bacilli 6 times in 10 teeth examinations; and Koener (Tellier) twice in 20 teeth. Moeller,¹ in the *materia alba* of 41 non-tubercular children, found Koch's bacilli 6 times. In a group of 194 children, with pulmonary affections, 133 showed dental caries, 14 of whom showed tubercle bacilli in the carious material. In this same group of 194 children, 182 showed appreciable amounts of *materia alba*, 35 of whom showed tubercle bacilli. Emmerich² came to somewhat different conclusions. He believes that the *materia alba* of uncared-for mouths is not an important nursery or reservoir for tubercle bacilli. His method was direct microscopic examination alone. He studied in this way smears from the mouths of 398, presumably non-tubercular, children and found acid-fast rods but once. In another group, of 56 children of the same age (six to fifteen years) but obviously undernourished, no acid-fast bacteria were found. The same result was obtained in an examination of 11 patients suffering from frank pulmonary or oral tuberculosis and in the exudates of whose lesions tubercle bacilli had been recently found.

Zilz³ examined material from carious teeth of school children of various ages. Direct microscopic examination of the sediment after antiformin treatment showed acid-fast bacteria in 3 per cent of cases, while guinea-pig inoculations with the material gave 40 per cent of positive results.

DeVecchis⁴ injected 12 guinea-pigs with the products of dental decay, 5 of which died of tubercular peritonitis. Kramer and Downing injected a small amount of débris or pus from under the free margin of the gum, from each of 50 patients showing tubercle bacilli in their sputum, into 50 guinea-pigs. Twelve of these (24 per cent) developed tuberculosis. Of the 12 patients corresponding to these guinea-pigs, 11 were far advanced and 1 moderately advanced. Gautier⁵ examined 48 teeth for tubercle bacilli. Ten of these teeth were from healthy adults and none yielded tubercle bacilli; 8 of the teeth were from adults with healed or latent tubercular lesions and only 1 of the 8 yielded tubercle bacilli; 30 teeth were from 20 children ranging in age from five to ten years, all suffering from cervical adenitis—and tubercle bacilli were recovered 5 times.

¹ Die staedtische Schulzahnklinik, ein Hilfsmittel zur Bekaempfung der Tuberculose, Verh. V. internat. zahnaerztl. Kongresses, 1909, 2, 479.

² Deutsch. Monatsschrft. f. Zahnk., 1922, 40, 143.

³ XVII Internat. Congr. Med., London, Sect. Stomat., 1913.

⁴ Dent. Cosmos, 1915, 57, 738.

⁵ La Presse dentaire, 1922, 24, 14.

The studies, to which reference has just been made, seem to show quite conclusively that tubercle bacilli may occasionally be found in the mouths of tubercular patients. Apart from the danger to which the dental operator is thereby exposed or to which other patients of that operator may be exposed unless instrument sterilization is effective, the intra-oral presence of tubercle bacilli exposes the tubercular patient, himself, to secondary localizations of the disease. A case reported by Doutrelpont¹ serves as an illustration. The patient, already suffering from pulmonary tuberculosis, had a tooth extracted. Instead of the usual uneventful healing, a tubercular lesion developed at the site of the extraction. Similar cases have been reported by Rethi,² by Walter,³ by Goure⁴ by Erhardt,⁵ by Zilz⁶ and by Ivy and Appleton.⁷ Another case reported by these latter authors indicates that the site of the oral localization was determined by the fact that the patient had for some time worn a bridge whose abutment teeth gradually loosened and eventually fell out. Such a history means a periodontal wound of long duration, onto which tubercular sputum from the affected lungs was sown.

The Oral Tissues as a Portal for Tubercular Infection.—There exists no unanimity of opinion regarding the pathway by which Koch's bacillus generally gains access to the body. The dominant thought today is that in at least most cases of pulmonary phthisis the microorganisms enter directly with the inspired air. There exists, however, an active school, in which Calmette probably is most prominent, which holds that the initial portal in almost all cases is the intestinal canal. Some experimental work has been done which quite definitely shows that tubercle bacilli may enter the system through oral lesions. It must be kept in mind that this portal is only a possible one and that probably in the vast majority of cases entrance is effected elsewhere. There is a wealth of published material bearing on this subject, which after all it must be remembered, is but one aspect of the general problem of oral infectious foci. Cornet⁸ exhibited at the Eighteenth Kongress der deutschen Gesellschaft fuer Chirurgie, dogs whose incisor teeth had been mutilated with a blunt instrument. Into these defects tubercle bacilli had been introduced. Enlargement and caseation of the mandibular and cervical

¹ Ueber Haut- u. Schleimhauttuberkulose, Deutsch. med. Wehnschr., 1892, p. 1033.

² Wien. med. Presse, 1893, No. 19.

³ Therap. Monatshfte., 1895, Feb.

⁴ Rev. de stomat., 1905, 12, 531.

⁵ Deutsch. med. Wehnschr., 1911, p. 124.

⁶ Tuberkulose der Mundhoehle, Wien., 1912, Case II.

⁷ Jour. Am. Med. Assn., 1923, 81, 1483.

⁸ Berl. klin. Wehnschr., 1889, No. 12-14.

lymphatics resulted. In another series of experiments¹ Cornet introduced tubercular sputum into gingival pockets with blunt and pointed instruments. After about three weeks an ulcer appeared, although in some instances no change of any kind was recognizable at the site of inoculation even after eight weeks. Regularly, however, the submental, sublingual, and cervical lymph nodes especially on the inoculated side, underwent caseation. Later tubercles were to be found in part of the lungs and finally also in the spleen. The bronchial lymph nodes were only slightly or not at all caseated. Joseph Mendel² introduced virulent bovine tubercle bacilli into the dental pulp of the ape. A very severe form of generalized tuberculosis rapidly followed. In 1921, Kramer and Downing scarified the gums with a lancet and into this lesion they introduced relatively massive doses of tubercle bacilli; 10 guinea-pigs and 5 rabbits with human strain and 5 rabbits with bovine strain. All of the 10 guinea-pigs developed tuberculosis; none of the 5 rabbits inoculated with the human strain developed the disease, while all 5 of the rabbits inoculated with the bovine strain showed clear evidence of tubercular infection.

The impossibility of adequate control and of following any single case throughout its history renders clinical demonstration in man of systemic tubercular infection entering *via* oral lesions very difficult. In the first place, acid-fast bacteria have been found in the saliva of non-tuberculous persons.³ Cook⁴ reported 11 cases in which the acid-fast bacteria were demonstrated in the mouth, in 6 of which no clinical evidence of tuberculosis was obtainable.

From the clinical stand-point, Neisser⁵ reported a case of tuberculosis of the epididymis secondary to a primary tuberculosis of the oral mucosa. Tellier⁶ describes a case of tubercular meningitis ascribed to a "dental phlegmon." Moeller⁷ cites a case of pulmonary tuberculosis which he regards as probably originating *via* a carious tooth.

Partsch⁸ and many others are convinced that the majority of cases of tubercular submaxillary and cervical adenitis in children is due to extension from primary oral or dental lesions. Cobbett,⁹ on the other hand, regards it as "probable that the *tonsils* afford

¹ Die Tuberkulose, Wien., 1907, p. 133.

² Rev. de stomat., 1920, No. 2.

³ Loebs: Inaug. Diss., Freiburg, i. Br., 1894; Hoeller: Ztschr. f. Hyg. u. Infekt., 1889, 32, 211.

⁴ Dent. Rev., 1899, 13, 97.

⁵ Zilz, 1912, p. 68.

⁶ XIII Congr. internat. de méd.

⁷ München. med. Wchnschr., 1910, No; 2, p. 80.

⁸ Deutsch. med. Wchnschr., 1904, No. 39.

⁹ Causes of Tuberculosis, 1917, p. 590.

the main portal of entry for the tubercle bacilli which reach the cervical glands." This view, it seems, is the more generally held one today. Montigel¹ examined in 29 cases, the carious teeth of children with tuberculosis of the cervical lymph nodes, in reference to the presence of tubercle bacilli. Only in one case did he succeed in demonstrating an acid-fast bacillus, which did not prove pathogenic for guinea-pigs and which was thicker than the true Koch's organism. It may have been Petri's milk bacillus. Gautier,² as noted above, examined 30 teeth from 20 children, all suffering from cervical adenitis and recovered tubercle bacilli 5 times.

It is interesting to note, that bacilli of the bovine type play a very important part in tubercular cervical lymphadenitis. Cobbett³ estimates that, taking all countries together, about 50 per cent of these cases are due to bovine bacilli, if all cases irrespective of age be included. Under the age of five years the percentage probably rises to about 75. This is associated with the predominance of a milk diet in early years.

Differential Diagnosis.—The differential diagnosis between tuberculosis, syphilis and epithelioma of the soft parts of the oral cavity is difficult. The situation is further complicated by the fact that two or all three of these diseases may synchronously manifest themselves in the same mouth. An identical difficulty is encountered in the case of the mucosa of the *cervix uteri*. The tubercular ulcer is irregular, usually rather shallow, with slightly undermined but not elevated margins, with little or no induration. The surface is characteristically granular, covered with reddish and yellowish elevated points, with occasional yellowish streaks. Pain is a fairly constant feature, especially early in the disease (Ivy).

A positive Wassermann, the presence of unmistakable syphilitic lesions on the skin or mucosæ, a history of exposure, the demonstration of *Treponema pallidum* in the discharge of the lesion in question and rapid retrogression of this lesion under anti-syphilitic treatment suggest the diagnosis of syphilis. But even the fulfilment of all these conditions does not exclude the possible presence of tuberculosis, as the following case shows. Zilz⁴ reported an instance where a lingual syphilitic plaque was transformed by infected sputum into a tubercular ulcer.

The inability to demonstrate the specific microorganisms of syphilis or tuberculosis in the discharge from the lesion in question,

¹ Ash's Monthly, 1911, p. 502.

² La Presse dentaire, 1922, 24, 14.

³ Loc. cit., p. 601.

⁴ Sitzungber. d. niederrh Ges., 1886, 5, 19.

a negative Wassermann, a negative history of syphilis, the absence of unmistakable symptoms of syphilis, the absence of nasal, pharyngeal, laryngeal, and pulmonary signs of tuberculosis and a history of leukoplakia suggest the possibility of epithelioma. The presence of a history of syphilis cannot rule out the possibility of epithelioma. In fact there is a considerable body of clinical evidence for the view that syphilis definitely predisposes to leukoplakia and epithelioma of the tongue. Similarly, the site of a long-standing lupus may undergo carcinomatous degeneration. A definite diagnosis of epithelioma can be most readily made by histological examination of excised tissue. But recourse to this method is strongly contraindicated by the fact that in case the condition be actually epithelioma, metastases to the regional lymphatics is greatly favored thereby. This seriously diminishes the chances of recovery.

When the lesion affects the maxilla or mandible it is difficult to differentiate between syphilis, actinomycosis, sarcoma, and tuberculosis. What has been said above regarding the recognition of syphilis applies here. The gumma of the tertiary stage is the lesion *par excellence* in this field.

Agricultural occupation and the demonstration of the "sulphur granules" in the discharge, together with the absence of regional lymphatic involvement, suggest actinomycosis.

The radiographic findings in sarcoma are often in themselves sufficiently characteristic for diagnosis.

Oral Prophylaxis and Tuberculosis.—There are a number of phases of the relation of oral prophylaxis, using this term in its broadest sense to include orthodontic readjustments, prosthetic appliances and operative procedures, to tuberculosis. In the first place by establishing or maintaining oral health, the mouth may easily be excluded as a possible portal for systemic involvement.

The likelihood of oral tuberculous lesions developing, secondary to infection elsewhere in the body, may be reduced to a negligible minimum by careful and systematic oral hygiene. And finally, there seems to be a well-defined impression among clinicians that oral health favorably influences the course of pulmonary or systemic tuberculosis. *Per contra*, oral disease exerts an unfavorable influence.

Some of the most trying and serious complications of pulmonary tuberculosis, such as fever and cavity formation, are ascribed to the secondary invasion of the lesions by ordinary pyogenic bacteria, *viz.*, pneumococci, streptococci, staphylococci and *Micrococcus tetragenus*. All of these microorganisms thrive in the neglected

mouth, and their aspiration would be certain. Pilot, Davis and Shapiro¹ assert that fusiform bacilli and spirochetes (Vincent's organisms) cause secondary infection in pulmonary tuberculosis. They are usually responsible for the fetid expectoration in bronchiectasis and gangrene. Prevention of these putrid infections depends on proper care and hygiene of the mouth. See also in this connection Sinclair.²

Summary.—Tubercular infection of the oral cavity is very rare. It may be primary, or secondary to tuberculosis of some other part of the body, usually of the lungs. Most oral tubercular lesions are of this secondary type. Primary, oral tubercular lesions may very rarely serve as a portal for tubercle bacilli to enter the human body. It is possible that some cases of tubercular, cervical lymphadenitis may arise in this way. The maintenance of oral health naturally diminishes the chances for the occurrence of these primary, oral foci of tubercular infection.

Neglect of the health of the mouth, dental caries, and periodontal lesions predispose to the secondary localizations of tuberculosis in the mouth. The maintenance of oral health in the tubercular practically eliminates the possibility of the occurrence of these secondary manifestations.

A still more cogent reason for the maintenance of oral health in the tubercular is that oral sepsis favors the invasion of pulmonary tubercular lesions by pyogenic bacteria and Vincent's organisms. These secondary invaders seriously complicate and aggravate the general and local condition of the patient. The maintenance of oral health is strongly indicated in the tubercular. The value of an efficient masticatory apparatus is self-evident in a class of patients where recovery is so influenced by the nutritional condition. And finally oral infection by putting an added drain upon the resources of the patient, who needs all these resources to combat the tubercular infection, may serve as the proverbial last straw.

SYPHILIS.

*Pallida mors aequo pulsat pede pauperum tabernas
regumque turris.* — Horace, C., I. iv.

The social import of syphilis is conspicuous and widely recognized. Its seriousness to the individual and to the family is fairly well appreciated by the laity. The cause of this disease is a spiro-

¹ Am. Rev. Tuberc., November, 1923.

² Ibid., 1920, 4, 201.

chetal form, discovered by Schaudinn (1905) and named either *Spirochæta pallida* or *Treponema pallidum*. It is a cylindrical, spirally wound filament, with pointed ends, 0.25 to 0.3 by 6 to 14 μ . The spiral amplitude is 1 μ and regular. The spiral depth is 0.5 to 1 μ and very constant. The number of curves in the spiral varies from 6 to 30. The entire spiral presents one or more slight, undulating curves. Although highly motile, flagella are absent. The type of motility is very characteristic, consisting in a rotation around the long axis of the organism with a gliding backward and forward. Sometimes the movement depends upon a bending of the body in its entirety. Usually the same individual can be kept under observation with no difficulty in the same general part of



FIG. 86.—*Treponema pallidum* appearing as bright refractive body on a dark field as shown by India ink or ultramicroscope.

the microscopic field. The organism stains only satisfactorily with special dyes, *e. g.*, impregnation with silver nitrate, as in Levaditi's method, or Giemsa. It is perhaps best and easiest observable in the living state by means of dark-field illumination. This method depends upon directing the rays of light which illuminate the microscopic field so that they run approximately at right angles to the optical axis of the microscope instead of running parallel with that axis. The result is a dark or black field in which suspended objects are intensely illuminated. The principle operative is that which permits us to see the dust particles suspended in the air when a beam of sunlight traverses a fairly dark room. When examined by this method the microorganism of syphilis tends to

retain its coils while the ordinary mouth spirochetes tend to lose their coils after death. The ability of *Treponema pallidum* to penetrate the placenta is well known and the ability of penetrating intact mucous membranes is also commonly assigned to it, although it is difficult to conceive how that intactness could be proved. This microorganism is a strict anaërobe and is very difficult to isolate and cultivate artificially in pure culture. The medium usually employed consists of a diluted serum or ascitic fluid containing a piece of fresh sterile rabbit kidney or testis. It is a very delicate organism, absolutely unable under natural conditions to propagate or even to survive for more than a brief period outside the human body. It is rapidly destroyed by drying, sunlight or temperatures much below that of the human body. It is very sensitive to chemicals, including soap and water.

Syphilis is under natural conditions an exclusively human disease, although it has been experimentally transmitted to some primates and to rabbits. It is usually (in about 90 per cent of cases) contracted by sexual intercourse. In about 10 per cent of cases the infection occurs in other ways, known collectively as extragenital. The human species seems to be almost or quite universally susceptible. The ravages may be greater among long-isolated primitive peoples when first brought in contact with "civilization" and the disease may run a severer course when infection is acquired from a strain other than that one common for the patient's race. For instance, syphilis has exhibited a fulminating character when contracted by European sailors from oriental sources. No one can count on that chance of individual resistance which is so well known in the case of diphtheria and scarlet fever and which is to be observed in such infections as tuberculosis, typhoid fever or even bubonic plague, typhus, and Asiatic cholera. A further and related fact of much importance is that spontaneous, *i. e.*, without treatment, recovery probably never occurs, although long-latent periods when the infection is not clinically apparent, are not uncommon. Of course, the patient during such a latent period may succumb to some intercurrent cause. Recovery from syphilis does not confer immunity upon the individual. Indeed, it has been facetiously observed that the only way it may be known that a patient has recovered, is his contracting the disease anew. This paragraph contains three generalizations which may be succinctly stated as follows: All non-syphilitic human beings are susceptible to syphilis; spontaneous recovery does not occur; and recovery does not confer immunity or heightened resistance.

The diagnosis of syphilis is made by the demonstration of the causative microorganism in the lesions or in the discharges therefrom, by the physical appearances of the lesions, by the history and general condition of the patient and by certain serological and spinal fluid examinations, of which the Wassermann test is the best known.

It is customary to recognize three stages of the disease: Primary secondary and tertiary. There also exist other symptoms which are spoken of as parasymphilitic, metasymphilitic or quaternary. In this latter group fall such conditions as *tabes dorsalis* or locomotor ataxia, general paresis, and many cases of non-traumatic aortic aneurysm. Syphilis also is one of the most fertile causes of spontaneous abortion, premature birth, and sterility. It has been regarded as an important factor in *leukoplakia buccalis* and the consequent *carcinoma*.

The characteristic lesion of *primary* syphilis is the hard chancre. This is usually located on the genitals and not rarely in females is the patient unaware of, or unimpressed by, its existence. It may occur, however, on any part of the body surface, including the fingers, lips, gums, tongue, and tonsils. The following description is taken from Osler:¹ "The initial sore appears within a month after inoculation, and it first shows itself as a small red papule, which gradually enlarges and breaks in the center, leaving a small ulcer. The tissue about this becomes indurated so that it ultimately has a gristly, cartilaginous consistence, hence the name, hard or indurated chancre. The size attained is variable, and when small the sore may be overlooked, particularly if it is just within the urethra. The glands in the lymph district of the chancre enlarge and become hard. Suppuration both in the initial lesion and in the glands may occur as a secondary change."

Usually within three months after the appearance of the hard chancre in the typical, untreated case, the symptoms of the *secondary* stage manifest themselves, indicating the general distribution of the infectious agent. Among these symptoms, which include fever of very variable character, anemia and arthritis, the most prominent are those involving the cutaneous and mucous surfaces. The so-called mucous patches are of professional interest to the dentist. It will aid in understanding them if we regard them as essentially no different than the cutaneous syphilides—modified by such factors as the thinner epithelial covering, the presence of moisture and secondary bacterial invasion. ". . . the throat and

¹ Practice of Medicine, 6th ed., 1906, p. 267.

mouth become sore. The pharyngeal mucosa is hyperemic, the tonsils are swollen and often present small, kidney-shaped ulcers with grayish-white borders. Mucous patches are seen on the inner surfaces of the cheeks and on the tongue and lips.¹ The tonsillar and oral mucous patches show an abundant bacterial flora, often including Vincent's organisms so that the condition may be considered to be merely one of Vincent's infection. Microscopic examination, *e. g.*, dark-field illumination of the exudate from these patches may show *Treponema pallidum* but its differentiation from common oral spirochetal forms, *e. g.*, *Treponema microdentium*, is so difficult that it is probably unwise to put much reliance on this method of diagnosis. In a study of the healed syphilitic plaques of the mouth in 28 cases Scheele² became very positive that in a significant majority *Treponema pallidum* was absent, and that in no case was the evidence for its presence adequate to justify any other than the Scotch verdict of not proven.

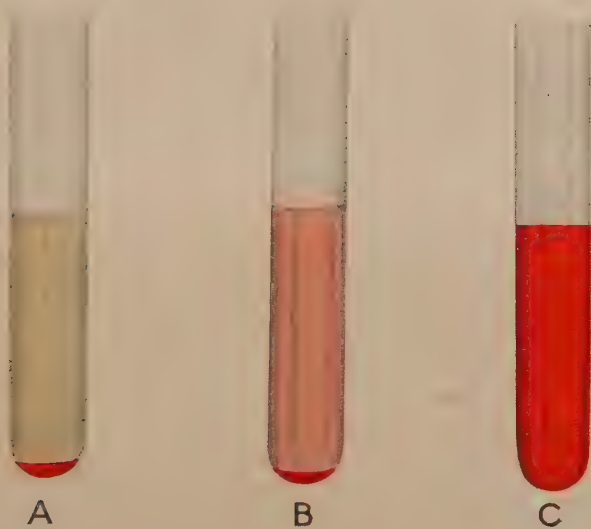
The most characteristic lesion of *tertiary* syphilis is the *gumma*, which may be localized anywhere in the body—including the tongue, salivary glands and jaw bones. The perforation of the hard palate into the nasal fossa and the destruction of the external nose sometimes seen in syphilitics belong to this category. Gummata "vary in size from small, almost microscopic bodies to large solid tumors from 3 to 5 cm. in diameter. They are usually firm and hard, but in the skin and on the mucous membranes they tend to break down rapidly and ulcerate. On cross-section a medium-sized gumma has a grayish-white, homogeneous appearance, presenting in the center a firm, caseous substance, and at the periphery a translucent, fibrous tissue. Often there are groups of three or more surrounded by dense sclerotic tissue." The syphilitic gumma belongs to the pathohistological category of the infectious granulomata.

The Wassermann Reaction.—This test has proved to be of the highest utility in the diagnosis of syphilis. The theoretic principles on which it rests are not hard to comprehend. Its performance, on the contrary, from the preparation of the "antigen" and in the preliminary and control titrations, is very complicated and difficult. It requires the most scrupulous, painstaking and intelligent attention to every one of its multitudinous details. And finally, the definitive interpretation of the results calls for long, rich experience and clinical association. It is not a test in which one can perfect himself quickly. A negative result does not necessarily exclude the possibility that syphilitic infection actually exists, although a

¹ Osler: *Loc. cit.*, p. 268.

² *Med. Klin.*, 1921, **17**, 1176.

PLATE V



Wassermann Reaction. (Kendall.)

A, positive; *B*, partial; *C*, negative reaction.

Note undissolved blood corpuscles in *A*, partial hemolysis in *B*, and complete hemolysis in *C*.

positive result with correct technic is strong evidence for the presence of syphilitic infection. The test usually is negative when made before the hard chancre appears. It may become positive during the first week after the appearance of the initial lesion. Positive Wassermanns have been repeatedly claimed to be the rule for a rather large number of totally distinct diseases, including Vincent's infection (*q.v.*). The consensus of opinion, however, at the present time is that such positives are really false, due to technical and largely avoidable errors; that, in short, with proper technic the Wassermann is extremely rarely positive in diseases other than syphilis with the sole exception of yaws (*frambesia*). This is caused by a spirochete morphologically indistinguishable from that of syphilis, and known as *Treponema pertenue*. *Frambesia* is a tropical disease and in the north temperate latitudes is not a confusing factor.

Wassermann and the earlier workers who devised this test, did so in the belief that it was a genuine complement-fixation reaction. They sought to apply the principles which had been developed by Bordet and Gengou in a rather academic research upon the unity or multiplicity of complement (alexin). Parenthetically, we must note that the clinical success of the Wassermann illustrates the great value of so-called academic researches and of theoretical speculations, *i. e.*, "working hypotheses," whose elaboration leads to sounder conceptions and material advantages.

Wassermann was looking for a specific amboceptor in the patient's serum, which in the presence of the corresponding antigen would quantitatively bind complement. The antigen is the specific cause of the disease, in this case *Treponema pallidum*. In order to secure an abundant supply of these microorganisms, he made use of the liver of a syphilitic fetus, which is crowded with enormous numbers of the spirochetes. Wassermann argued that if the patient had syphilis and if amboceptor was among the antibodies consequently formed, then the patient's serum plus the specific antigen would bind complement. This result would be rendered visible to simple naked-eye inspection of the tubes by the use of a hemolytic system minus complement. If the patient's serum contained amboceptor (*i. e.*, if the patient was syphilitic), then the union of antigen and complement would have been effected and hemolysis would not have occurred (Plate V). The erythrocytes would have remained intact and fallen to the bottom of the tube. The supernatant fluid would not have been discolored reddish because hemoglobin was still retained within the intact erythrocyte. The absence of hemo-

lysis under these conditions would indicate the presence of anti-syphilitic amboceptor in the patient's serum capable of binding complement, *i. e.*, the absence of hemolysis indicates a *positive* reaction. If the patient's serum did not contain amboceptor (*i. e.*, if the patient was not syphilitic), then the union of antigen and complement would not have been effected. Complement would have left to complete the hemolytic system and hemolysis would have resulted. The erythrocytes would have been disintegrated and the liberated hemoglobin would have diffusely colored the contents of the tube reddish. The presence of hemolysis indicates a *negative* reaction.

Wassermann's surmises were amply confirmed and justified, although further studies soon showed that he was not working with a genuine complement-fixation test. Instead of an extract of the causative microorganisms being indispensable as antigen, it was found that many substances having nothing whatever to do with *Treponema pallidum* or syphilis could be used as antigen. Some of these substitutes have proved even more satisfactory than the true antigen which at the present time has been quite given up for routine work. Such substitutes are derived from a great variety of sources, including the liver and heart not only of the normal (non-syphilitic) human being but of various domestic and laboratory animals, naturally immune to syphilis. These substitutes are probably essentially extracts of tissue lipoids. This fact that several substitutes for *Treponema pallidum* can advantageously be employed as antigen, renders the Wassermann reaction *not biologically* specific, although when properly done it is *clinically* specific. Inasmuch as the "antigen" customarily today used in the Wassermann is not a true antigen in the immunological sense of the word, doubt is naturally cast upon the original assumption that the substance in the patient's serum, which together with the "antigen" binds complement, is a true amboceptor. It is perhaps wiser to envisage the mechanism of the Wassermann, none too definitely but somewhat as follows: Due to the presence of syphilitic infection, some change is induced in the serum which together with the "antigen" determines the adsorption of complement. Surmises have been offered as to the nature or character of that change, but no view so far proposed has received general acceptance. The patient's blood serum is not the only constituent which may be used for the Wassermann. The spinal fluid is frequently used and may yield a positive although the serum yields a negative result, especially in cases of neurosyphilis. Positive results have been obtained

by Kolmer and Klauder using the exudate expressed from the chancre, and these same authors¹ have reported 1 instance (out of 20 cases) where a weakly positive reaction was obtained with saliva. This patient presented a profuse eruption of the buccal mucosa of the secondary stage.

The results of the Wassermann reaction are customarily reported as, four plus (+++), three plus (+++), two plus (++), one plus (+), doubtful ($\pm$), and negative. These represent approximately the varying degrees of hemolysis, which indicate varying degrees of complement-binding property of the patient's serum.

++++ = complete inhibition of hemolysis = strongly positive.

+++ = 75 per cent inhibition of hemolysis = moderately positive.

++ = 50 per cent inhibition of hemolysis = weakly positive.

+ = 25 per cent inhibition of hemolysis = very weakly positive.

$\pm$ = less than 25 per cent inhibition of hemolysis = doubtful reaction.

- = complete hemolysis = negative reaction.

Syphilis in Its Relation to Dentistry.—In the first place, syphilis has been innocently contracted by dentists during even the most ordinary oral operations, as scaling the teeth or taking an impression. The infection enters usually through a cut, abrasion, or scratch on the skin of the finger. The wound may be due to a slip of the bur or instrument or it may be so minute that the dentist himself is unaware of its existence. In case such an accident is suspected of having happened, a bit of information may prove of value. The institution of active antisymphilitic treatment soon after contact with a known, even florid, case of syphilis may succeed in completely preventing infection with *Treponema pallidum*. At times the dentist by means of infected instruments has carried syphilitic infection from one patient to another. The possibility of this is one of the most cogent reasons for the sterilization of instruments.

From the danger of infection to the dentist and his clientele the patient is most menacing who presents the oral mucous patches of the secondary stage. These lesions often teem with the spirochetes. The hard chancre of the soft tissues about the mouth is also a source of danger, while the ordinary tertiary lesions are least dangerous. It must be remembered by the practising dentist that syphilis is no respecter of persons. It is common among all grades, groups, races and nationalities of the community; among rich and poor, educated and illiterate, old and young, among all

¹ Jour. Am. Med. Assn., June 11, 1921, vol. 76.

occupations and professions from the oldest where it is expected to the most recent. At the same time the dentist must not be hasty in positively diagnosing syphilis or in communicating suspicions to the patient or to the patient's family or friends. The subject on account of the social stigma attaching is delicate and the utmost tact, as well as honesty, is required. No general rule can be given, applying to such situations but it would seem wise to refer the patient to his physician and then personally communicate your own observations to that physician.

The syphilitic patient needs dental care and should not be denied it by the mere fact of being syphilitic. It is well to remember that in the case of infectious disease, the patient is more dangerous to his fellowman when the condition is unrecognized than when it is. The operative habits and precautions of every dentist as well as the sterilization of his instruments should be so ordered that the chance of contracting or transmitting any type of infection is reduced to a minimum. Under these conditions the recognized syphilitic patient can be treated with safety to the dentist and to his other patients.

The joint aid of the dentist is desirable in the treatment of such syphilitics as are being subjected to a course of mercurialization. In the older days the administration of mercury was often pushed until countermanded by the development of a severe stomatitis. Then mercury was suspended until the patient's oral condition had improved, and mercury was resumed. This alternation was continued as long as it was held desirable. Such a procedure is crude and rather heroic. At the present time, it is well-known that the administration of mercury can be carried on more intensively in patients whose mouths are first put in a healthy state and who receive concomitant oral attention. Roots, questionable teeth, and calcareous deposits should be removed. The trauma offered to the gingivæ by fillings, crowns and bridges should be relieved. The patient's close and constant coöperation is required. In such ways the dentist can materially aid in general antisiphilitic treatment.

Syphilis has many oral manifestations, some peculiar to this disease and some also exhibited by a variety of other conditions. The strictly dental defects form the subject of an extensive report by Cavallaro.¹ We shall content ourselves with a bare and partial enumeration. The most classic stigma of oral syphilis is probably the enamel and dentinal hypoplasia which forms one unit of

¹ *Dent. Cosmos*, 1908, vol. 50; 1909, vol. 51.

Hutchinson's triad. This is pathognomonic of congenital syphilis. The hypoplasia by itself is not pathognomonic but is common as a sequel to any of the exanthemata. Pasini¹ found the *Treponema pallidum* in the developing dental tissues of a syphilitic fetus (Fig 87). The microorganisms were present in very small numbers at the base of the dental papilla, scattered irregularly in the intercellular substance or upon the endothelium or in the walls of the bloodvessels. At the apex of the papilla the parasites are present in much greater numbers. They are very scarce in the dental sac. Confirmation of these observations was announced by Cavallaro² and by Koehler.³

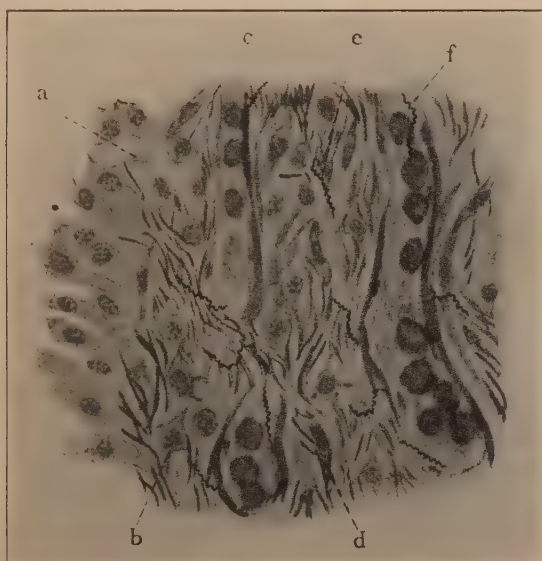


FIG. 87.—*Treponema pallidum* in the dental pulp of a developing tooth of a syphilitic still-born. *a*, Odontoblasts; *b*, polygonal cells; *c*, round cells; *d*, fusiform cells; *e*, *Treponema pallidum* in the gelatinous connective tissue of the papilla; *f*, *Treponema pallidum* in the lumen of a capillary bloodvessel, partially embedded in the endothelium. (Pasini.)

The lips probably afford the most frequent extragenital site for the primary chancre. Reference has already been made to the oral mucous patches of the secondary stage and to the gummata and palatal perforation of the tertiary. The tongue during this stage

¹ VI Congresso Stomatologico Italiano, October 3-5, 1908; *La Stomatologia*, 1908, **7**, 61; *Oesterr. Ztschr. f. Stomat.*, 1909, **7**, 97.

² *Dent. Cosmos*, 1908, **50**, 1325, and 1909, **51**, 181; *L'Odontologie*, Dec. 15, 1909, vol. **42**.

³ *Deutsch. Monatschr. f. Zahnk.*, 1913, **31**, 47.

is affected, *e. g.*, gumma and sclerotic glossitis. Leukoplakia and carcinoma have been mentioned as phenomena of the late syphilitic period. Tabes dorsalis presents several symptoms which may come to the attention of the dentist.¹ They present involvement of the nervous system. Neuralgiform pains may occur in the field of the fifth cranial nerve, simulating dental neuralgias. Attacks of salivation are sometimes associated with these trigeminal disturbances. Anesthesia and paresthesias permit the patient to bite unconsciously the cheeks, lips and tongue. The dental pulp, though vital, at times loses its sensitivity and also painless extraction without an anesthetic is possible. The patient may describe peculiar oral sensations, as if the teeth were pressed together or drawn out in length. Gustatory disturbances are noted, such as loss of taste in the anterior half of the tongue or unpleasant impressions. Perfectly healthy teeth, especially in the maxillæ, may be painlessly exfoliated without alveolar suppuration. Atrophy of the alveolar process has been noted in tabetics, although it is not known whether as an ordinary sequel to, or the cause of, the exfoliation. Necrosis of the jaw bones with the painless formation of sequestra occurs. These bones become brittle and spontaneous fracture is known. Dental extractions are thereby complicated in these patients. Paralysis, atrophy and ataxia of the masticatory muscles have been described, as well as various disturbances of the temporo-mandibular articulation and the very rare, unilateral atrophy of the tongue.

Usually associated with the exfoliation of the teeth and the necrosis of the jaw bones, though also independently, there is found a perforating, anesthetic ulcer. A type of this ulcer is Fournier's "mal perforant buccal . . . a torpid, painless, little bleeding ulcer situated on the alveolar process (especially in the molar region) which occasions the softening and resorption of the subjacent bone and, in the maxilla, perforation of the maxillary sinus."

It must not be forgotten that though syphilis is not an uncommon disease, its oral manifestations do not often come to the attention of the general practitioner of dentistry. Although the incidence of cases of syphilis contracted has probably not appreciably diminished in recent years, it is certain that all types of secondary, tertiary and quaternary symptoms and lesions are far more infrequent than they were a generation ago. This may be accounted for by the operation of at least two factors: (1) The laity are better informed and have a saner attitude; they apply for diagnosis

¹ Kron: Misch's Lehrb. d. Grenzgeb. d. Med. u. Zahnheilk., 1923, 1, 558.

and treatment earlier, *i. e.*, usually with the appearance of the chancre (although it is known that adequate antisyphilitic treatment instituted shortly after exposure can prevent even the development of the chancre); and they are more willing to carry through to completion the always protracted and exacting course of antiluetic treatment instead of stopping treatment with the disappearance of obvious symptoms. (2) The advance in medical science has rendered the physician better equipped to cope with this disease. The reliability and general use of the Wassermann reaction not only for diagnosis but also in conjunction with and for the control of treatment, permit the work on the individual patient to be done more intelligently than ever before; and the introduction of the spirocheticidal, organic arsenicals by Ehrlich, such as salvarsan and neosalvarsan, was an event of the very highest importance for the therapy of syphilis.

CHAPTER XXVII.

FOCAL INFECTION.¹

THE knowledge that chronic infection about the teeth, even though the patient be aware of no discomfort, does at times insidiously result in serious and even fatal lesions in other parts of the body, has been of very great influence in dentistry. The imminence of this possibility has called forth the "root-canal problem;" it has made the use of roentgenography indispensable; it has been responsible for some modifications in the technic of extraction and of local anesthesia; it has indirectly determined a more rational application of, and significant constructional improvements in, crowns, bridges and dentures; it has afforded a great stimulus to the oral hygiene movement; in brief it has required the dentist to broaden his outlook, to consider oral conditions not by themselves as isolated problems, but in their relations to the human body in its entirety. The concept of focal infection has been the most powerful single factor at work in directing dental thought and practice in the past fifteen years.

A bit of etymology may help in understanding this term. The word *focus* in Latin meant a *hearth*, a center from which heat and light radiated. In medicine, a focus is a center from which infectious microorganisms spread. Such a focus may be a geographical district in which a disease is endemic and which serves as a reservoir from which epidemics and pandemics take origin. Foci of this nature are known in the case of yellow fever, malaria, cholera, plague and typhus fever. We are not, however, now concerned with those centers of infection but with centers of infection within the individual. A concrete example will help us to understand the meaning usually connoted by "focal infection." A patient has suffered for some months or years with a tonsillitis. In the course of time, symptoms of heart trouble appear and death may rather rapidly follow. At autopsy "vegetations" are found on the leaflets of one or more of the valves of the heart and cultures from these lesions may reveal the same microorganism as was found in the tonsil. The microorganisms entered the body *via* the tonsil. At

¹ The following discussion should be read with the facts and ideas presented in Chapter X, Part II, clearly in mind.

first they may have been localized at this site but in time from this infected tonsil bacteria were given off into the lymph or blood stream and carried through the circulation to gain under certain conditions a foothold on the delicate endocardium. The tonsillar infection in this case was the *focus*. The secondary valvular localization was the metastatic infection. If an embolus implies a mechanical occlusion of a bloodvessel, then not all metastases of this type are embolic in nature. Not only may the bacteria themselves be disseminated from the focus but it is also possible that toxic products of their growth may be liberated and carried by the circulation to do damage elsewhere in the body. Another point of importance is that when the bacteria develop in the secondary or metastatic localization, this site in its turn may serve as a focus for the further distribution of the infection.

Most of the primary foci are located in the head. The conditions included as such are generally of a chronic nature: Tonsillitis, adenoid infections, accessory nasal sinus infections, otitis media, mastoiditis, "pyorrhea alveolaris," and periapical infections. Other foci are found in parts of the gastro-intestinal tract, especially the gall-bladder, appendix and possibly the cecum and colon. The genito-urinary tract is also of importance in this connection; the microörganism often involved is the gonococcus, localizing secondarily in the joints and on the heart valves and producing respectively a gonococcal arthritis or a gonococcal endocarditis. The following case clearly illustrates this.¹ A white male, aged forty-two years, had suffered with arthritis for many years. As he denied gonococcal infection attention was directed away from the genito-urinary organs and another focus was sought. The teeth and accessory nasal sinuses were roentgenographically examined. The tonsils were normal. All possible foci other than the prostate and seminal vesicles were eliminated. The prostate was enlarged and somewhat boggy, and the left seminal vesicle was slightly palpable. Four tubes of testicular agar were inoculated, and after seventeen days of subculturing, a pure culture of gonococci was obtained. The prostate is a common site of infection with gonococci, colon bacilli or other pyogenic bacteria. It is obvious that the primary foci are situated along surfaces directly communicating with the exterior.

The conditions described as metastases from the primary focus are numerous and variable. The classification given below is based on one compiled by Barker:² Diseases of the *locomotor system*:

¹ Hogan: Jour. Am. Med. Assn., 1925, **84**, 195.

² Jour. Dent. Res., 1920, **2**, 43.

Infectious arthritis (arthritis deformans), hypertrophic osteoarthritis, local osteomyelitis, septic osteitis, myositis and fibrositis (bursitis, etc.); diseases of the *respiratory system*: Sinusitis, laryngitis tracheitis, bronchitis, bronchial asthma, tonsillitis, embolic pneumonia, pleuritis; diseases of the *circulatory system*: Acute infectious endocarditis, subacute infective endocarditis (endocarditis lenta), pericarditis, myocarditis, cardiac irregularity, *e. g.*, extrasystolic arrhythmia, phlebitis and thrombosis, *e. g.*, cavernous, arterial hypertension, vertigo, arterial sclerosis, and angina pectoris; diseases of the *blood and blood-forming organs*: The secondary and primary anemias and Hodgkin's disease; diseases of the *digestive system*: Subacute or chronic gastritis with hyperacidity, subacidity or achlorhydria, gastric and duodenal ulcers, hepatitis, cholecystitis, pancreatitis, appendicitis; diseases of the *urogenital system*: Glomerulo-tubular nephritis, pyelitis, ureteritis and cystitis, toxemias of pregnancy; diseases of the *nervous system*: Multiple neuritis, chronic neuralgias, neurasthenias, psychoses, chorea, singultus, meningitis, encephalitis, and hemiplegia; diseases of the *eye*: Iritis, iridocyclitis, neuropathic keratitis, interstitial keratitis, uveitis, disturbances of accommodation, retino-choroiditis, retinal hemorrhage and blepharospasm; diseases of the *endocrine and metabolic systems*: Hyperthyroidism and undernutrition; and diseases of the *skin*: Erythema nodosum, herpes zoster, alopecia, lupus erythematosus, furunculosis, pruritus ani, and urticaria.

It must not be thought that every chronic localized infection is actually a focus but it is potentially a focus. Conversely all the instances of the pathological conditions listed in the above paragraph as secondary localizations are not exclusively ascribable to focal infection. Many factors are responsible for the successful metastasizing of a local discrete infection; but we are still largely in the dark concerning the identity, so say nothing of the evaluation of these factors.¹

An infectious focus, even though it be not genetically responsible for some particular remote lesion, may exert an unfavorable influence upon the course of the latter. Attention has been called to this phase of the subject in the case of diabetes mellitus, hyperthyroidism, tuberculosis, syphilis, and malignancy of the mouth, pharynx, trachea, larynx, esophagus, stomach and intestine. Adami's concept of subinfection (q.v.) is useful here. Bacteria are constantly throughout life passing into the lymph and bloodvessels, to be carried to distant parts, there normally to be destroyed. This

¹ Appleton: Dent. Cosmos, July, 1924.

destruction liberates bacterial proteins and endotoxins which are capable of locally setting up degenerative and necrotic processes with consequent fibrosis. There is no multiplication of bacteria in the tissues. The bearing of this idea in dentistry is obvious. From a quiescent, latent, oral focus or from an acute periapical or pyorrhetic process, *unusual* numbers of bacteria enter the lymph and blood stream. Their subsequent destruction, even though the body be strong enough to prevent their multiplication or to prevent manifest symptoms, will entail more numerous and more extensive focal degenerations and necroses throughout the tissues, with proportionate impairment of function. The cumulative effect of these insults would at best accelerate the development of that vague condition known as senility.

The evidence that chronic, local infection can be responsible for damage in remote parts of the body, *i. e.*, the evidence for the concept of focal infection, may be summarized as follows:

1. The same bacteria which are found at the focus have in some instances, been recovered from the remote lesions. The streptococci almost exclusively are of significance in focal infection as understood here.

2. The bacteria isolated from the focus when injected into susceptible animals will sometimes bring about a condition in the animals similar to that seen in the patient.

3. The elimination of infection at the primary focus is in some instances followed by amelioration or cure of the "secondary" condition. The failure of improvement in such a circumstance does not definitively weigh against the doctrine of focal infection. If bacteria have successfully colonized at the site of the "secondary" lesion, *e. g.*, in heart valve, their removal from the primary portal may prove unavailing.

4. The administration of autogenous vaccins prepared from bacteria isolated at the primary focus, may call forth symptoms at the sites of "secondary" localization, *e. g.*, in a joint.

5. When the primary focus is subjected to surgical trauma, as in dental extractions, there sometimes occurs an exacerbation of the "secondary" condition.

Because of the importance of focal infection, the question comes up frequently in dental practice. Here is a periapical infection with some local reaction. Its eradication without extraction is doubtful or problematical. The patient is in apparent good health. No history or evidence of possible secondary involvements is elicited. For functional and cosmetic reasons it is desirable to retain the

tooth. Is there any way of telling whether this primary infection will or will not be followed by metastases? The answer at the present time is: No, no such prognosis is possible. In deciding the procedure to be carried out in such a case the practitioner should keep in mind certain considerations.

In the first place, the microorganisms involved (the streptococci seem to be of prime importance) are pathogenic. The lesions produced in rabbits by strains from persons without systemic disease were found by Moody¹ to be identical in character with those produced by strains from patients with systemic disease. The fact that the former strains do not produce lesions quite as frequently as the latter indicates a difference between the strains only in degree of virulence. It is possible that the sojourn of the bacteria at the initial lesions serves to enhance their virulence. The period during which the microorganisms themselves are localized at their point of entrance, may also afford the opportunity for the sensitization of the patient in an allergic sense. In brief we cannot dismiss the streptococci of periodontal or periapical lesions as innocuous.

In the second place, it is a moot point whether infected periapical tissues can be sterilized with the tooth *in situ*; or if sterilized, it is uncertain how long that condition can be maintained. Bacteriological examination can at best show only the persistence of infection. Negative cultures cannot be depended upon as indicating sterility. Roentgenographic evidence of bone regeneration after treatment is sometimes obtained in the presence of infection. The vast majority of pulpless teeth, no matter what the treatment, will upon extraction with adequate precautions against contamination, yield growths of streptococci. The circulatory disturbance attendant upon the loss of the pulp in some way permanently lowers the local resistance. Even though a particular infection be eliminated, we have a *locus minoris resistentiæ* at which lymphogenous or hematogenous reinfection is more likely to survive than normally. In the words of Sir Arthur Keith² a pulpless "tooth is not a dead tooth, but it is one which is imperfectly nourished, unfit for hard wear, and liable to atrophy and infection." In the light of what we know today every pulpless tooth, to say nothing of one with a history of periapical infection, must be regarded with suspicion.

In the third place, it must be remembered that the actual and potential results of infection have so impressed medical thought that the consensus of surgical opinion today is that no focus of infection, if it can be eliminated, should be tolerated. The danger

¹ Jour. Infect. Dis., 1916, 19, 515.

² Lancet, 1924, 207, 741.

of focal infection is something besides the development of rather obvious clinical entities. If a patient presents himself with oral infection and an arthritis or an endocarditis or a cholecystitis or a gastric ulcer, it is usually easy, all things considered, to decide what to do. The other extreme, where the patient is youthful and except for oral infection in exceptional health, also offers little difficulty. The attitude indicated here is succinctly stated by Davis¹ in discussing diseases of tonsillar origin. "It should be realized that the safest position for the medical man to assume with respect to operations in general and especially with respect to the removal of organs is that of a progressive conservatism. It is safer to act upon clear indications when they arise, even though occasionally the consequences may be serious, than to indulge in the more or less wholesale removal of organs, whatever may be their supposed function."

A third category of cases lies intermediate between the two extremes to which reference has just been made. This intermediate group is numerically the largest and presents the greatest difficulty to the general practitioner. The patients are middle-aged or older. They are not in robust health; they are conscious of a loss of zest for life and work, although no definite, clinically recognizable, lesions can be found—except oral infection. The question arises as to the relation of that condition to their state of lowered, general, physical efficiency. It may aid in the understanding of such cases to recall Adami's concept of subinfection. The effect of low-grade, chronic focal infection need not be limited to a particular organ or part, as to a joint or a gall-bladder; but it may be more diffuse and generalized. Such a mechanism would theoretically account for the condition presented by this third category. In this connection it should not be forgotten that this group may be largely recruited from that identified above as youthful and in exceptional health. These are possibilities, they are harmonious with current views on infection, but it is extremely difficult or impossible to prove that these plausible "explanations" correspond with what actually happened or will happen.

Many attempts have been made to show that focal infection is at least a factor in such a patient's condition. Blood counts and urinalysis (see Section on Symptoms of Infection) give little or nothing of definite value. The uric acid content of the blood has been proposed by De Niord as an index for the presence or absence of focal infection. But it has been shown (Section on Symp-

¹ *Scientific Month.*, 1924, **19**, 379.

toms of Infection) that an hyperuricacidemia is present in so many other conditions that, even granting its reliability, its range of usefulness is narrow. Blood counts and the chemistry of blood and urine appear at most capable of indicating only the presence of chronic infection. They give no clue as to the site of that infection. Isolated attempts have been made to employ the several serological tests and cutaneous tests for hypersensitivity, toward the solution of this problem. The organisms or "antigens" employed would be autogenous and those isolated from one or another of several sites of chronic infection. Up to the present no method of general applicability has been evolved. The clinician and the patient want to know if a low-grade, chronic infection is contributing significantly to the patient's condition, they want to know the site of that infection and they further want to know what effect upon the particular condition may be expected to follow the removal of the infection. These questions cannot be definitely answered. In such circumstances, the most reliable, available guide is the experience which furnishes analogies with similar cases.

ELECTIVE TISSUE AFFINITY.

No discussion of focal infection could be considered adequate if it ignored that hypothetical attribute of the bacteria designated by Rosenow as elective tissue affinity. This means in its narrow sense that a streptococcus isolated from a given site will when opportunity offers tend to become localized at the same site in experimental animals or another individual. This localization is not absolutely determined but is claimed to happen more often than not. This phenomenon is well illustrated by a report of Haden.¹ Sixty-eight and two-tenths per cent of rabbits inoculated with cultures obtained from patients suffering from metastatic eye infection developed lesions of the eye; while only 14.8 per cent of rabbits inoculated with cultures from patients without eye disease, developed lesions of the eye. Rosenow in the interpretation of such data regards the important factors as resident in the bacterial cell. Most of his critics, who do not question or deny the validity of the data, look for the significant localizing factors in the tissues rather than in the bacteria.

Similar localizations or elective tissue affinities are not unknown in bacteriology. For example, the virus of acute rheumatic fever, whatever it may be, exhibits a preference for the joints and leaflets

¹ Arch. Int. Med., December 15, 1923.

of the heart valves; the meningococcus after a transient stage in the circulation settles in the meninges; the virus of mumps prefers the parotid and the gonads; and the directness with which *Bacillus mallei* (glanders) when injected intraperitoneally into the guinea-pig, seeks the testes, has long been used in diagnosis. Recently attempts have been made to demonstrate neurotropic, dermatropic, and viscerotropic strains of *Treponema pallidum*, showing tendencies to become localized respectively in the nervous system, in the skin, or in the internal organs.

The concept of elective tissue affinities represents at most an unessential refinement added to that of focal infection. The acceptance or rejection of Rosenow's view that certain streptococcal strains possess or acquire properties which tend to make them localize in certain organs or tissues, can exert little or no influence, whether positively or negatively, upon the general concept of focal infection.

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CHAPTER XXVIII.

CLINICAL DENTAL BACTERIOLOGY.¹

At present bacteriological methods are clinically advantageous in dentistry for diagnosis, for "checking-up" on the treatment, and to facilitate coöperation with the physician in cases in which oral infectious foci are suspected. To illustrate each of these applications, it may be pointed out that ulcero-membranous stomatitis (Vincent's infection) can only with certainty be diagnosed bacteriologically, that the field in which bacteriological methods find their widest use at the present time is in the "checking-up" of root-canal treatments, and that the presence of infection in a suspected case can only definitely be proved by appropriate bacteriological methods.

For convenience we shall limit our consideration of the clinical applications of bacteriology in dentistry to three types of conditions: (1) The affections of the periapical tissues; (2) the affections of the parietal periodontium, *e. g.*, "pyorrhea alveolaris," and (3) the affections of the oral mucosa, *e. g.*, the gingivitides and stomatitides. The bacteriological measures adapted to the rarer oral diseases, *e. g.*, thrush, noma, tuberculosis, syphilis, gonococcal stomatitis, and diphtheria, will not be described.

Before detailing the indications and the means for bacteriologically examining these conditions, some general remarks are in order. In the first place the examination may be cultural or microscopic, or both. Much valuable information can be obtained from the simple microscopic inspection of a stained or unstained smear made from the discharge from the lesion. The more elaborate anaërobic methods for routine clinical examination in dentistry may for the time be ignored. Rosenow's glucose brain broth (see *infra*) in tall tubes offers anaërobiosis adequate for the growth of tetanus bacilli.

CULTURE MEDIA.

The question of culture media is highly important. The medium must be chosen with an eye to the requirements of the micro-

¹ The bulk of this Chapter appeared in the Dental Cosmos for March 1924.

organism suspected. For example, if one is looking for the influenza bacillus of Pfeiffer or one of its relatives the medium must contain hemoglobin; if one is looking for the diphtheria bacillus, the use of Loeffler's coagulated horse-serum is desirable. The cultivation of the oral spirochetes and the fusiform bacillus is difficult and, as it entails anaërobiosis, it will not be described here. Those interested in this phase are referred to Weaver and Tunncliff,¹ Tunncliff,² and to Noguchi.³

The culture media may be liquid or solid. Nutrient infusion bouillon or nutrient agar-agar, with or without 1 per cent dextrose, may be employed as a base, to which are added small amounts (5 to 10 per cent by volume) of sterile native (unchanged by heat or chemicals) proteins, *e. g.*, whole or defibrinated blood (sheep, rabbit, horse or human), blood serum or ascitic fluid. It seems advisable to have the reaction of the medium adjusted to a pH of 7.6 to 7.8⁴

Fisk and Burky⁵ give the preparation of a "hormone broth," which permits a luxuriant growth of streptococci:

To 3 liters of water warmed to 60° C. they add 3 beaten eggs and 5 pounds of ground beef heart. To this warm mixture they add 2 liters of water containing the following dissolved materials: 50 gm. of peptone, 12.5 gm. of salt, 12.5 gm. of trypsinized peptone ("aminoids") and 50 gm. of gelatin. The entire mixture was gradually raised to the boiling point without stirring after the beef had started to coagulate. It was boiled from fifteen to twenty minutes. A small portion was filtered through filter paper and to the measured filtrate was added enough 10 per cent HCl to cause the maximum precipitation. HCl was added to the unfiltered portion of the mixture in the same proportion. It was boiled for a few minutes. The precipitation with HCl was repeated until no further precipitation occurred. When there was no further precipitate, the liquid was drawn from the coagulum and sterilized at twelve pounds of pressure for fifteen minutes.

The next day the supernatant fluid was siphoned from the sediment. The reaction was adjusted to pH 7.8 to 8 in the cold (0.25 per cent dextrose was dissolved in the mixture by boiling). It was poured into tall jars and allowed to settle until the supernatant fluid was perfectly clear when it was tubed and sterilized, in the autoclave with apparently no disastrous results.

¹ Jour. Infect. Dis., 1905, **2**, 446.

² Ibid., 1906, **3**, 148.

³ Jour. Exper. Med., 1912, **15**, 81, and **16**, 194.

⁴ Fennel and Fisher: Adjustment of Reaction of Culture Mediums, Jour. Infect. Dis., 1919, **25**, 444.

⁵ Jour. Infect. Dis., 1922, **30**, 128.

Rosenow¹ introduced a dextrose brain broth and a soft dextrose brain agar medium which have been found widely useful in the cultivation of the streptococci. Haden² gives a simplified, but not significantly modified, recipe for the preparation of these media.

Glucose brain broth:

Dehydrated "Bacto" nutrient broth	8 gm.
NaCl	8 "
Dextrose c.p.	2 "
Andrade indicator	10 cc
Distilled water	1000 "

The broth and salt are dissolved by heating. After cooling, the indicator and dextrose are added. The medium is tubed in 6 inches by 0.75-inch test-tubes so that the depth is at least 3.5 to 4 inches. Three pieces of calf brain, about 1 cm. square and 2 or 3 pieces of crushed marble are added to each tube. Sterilized for twenty minutes in the autoclave at 20-pounds pressure.

Soft glucose brain agar:

Add 7 gm. of powdered agar to 1 liter of the glucose brain broth. The calf brain and marble are added. Sterilized as above.

Crowe³ advocates for streptococcal isolation the following medium. About 1 liter of bullock's blood is strained through muslin; 750 cc of this are placed in a water-bath at 50° C., 250 cc of ordinary pepton-agar or trypsinized peptone-agar of usual reaction are melted and cooled to 50° C. These two constituents are mixed. One per cent of glucose is added. The mixture is kept at 50° C. while plates or tubes are filled in the usual way. At least 0.25 inch depth of the medium is necessary in each plate. A liter should make from 33 to 35 4-inch plates. A piece of blotting paper is inserted into the cover of the plate. The medium becomes semisolid. It is conveyed without tilting onto some form of level shelf and put in a steam sterilizer. (A hot-air oven will do provided water is present to keep the atmosphere moist.)

The temperature is raised to 65° to 70° C. and there kept for one or two hours, then raised to 85° or 90° C. On no account must the temperature read 100° C. The temperature is again raised to 85° or 90° C. on three successive days, making four days in all. The surface of the medium should be perfectly smooth and glossy. The blotting paper is removed; the plates are dried in an incubator and stored until used.

¹ Jour. Dent. Res., 1919, **1**, 120.

² Arch. Int. Med., 1923, **32**, 1828.

³ Jour. Path. and Bacteriol., 1921, **24**, 361.

Although the author would not discourage the use of these more delicately adjusted and adapted media, he has found ordinary extract broth or agar-agar (as long as very moist), surprisingly satisfactory for routine work in isolating streptococci. Where the media are to be prepared on a small scale, as in an ordinary practice, the process is simplified and rendered more economical in time and money by reliance on some of the dried powders to be obtained on the market, which require only the addition of water before tubing and sterilizing. In general, however, it is the opinion of the writer that the individual practitioner will find it wiser, all things considered, to purchase media in small quantities already prepared from some neighboring clinical laboratory or hospital. Likewise after the inoculations have been made by the dentist himself, the wiser policy to follow will be to send at once the tubes for incubation and examination to some professional bacteriologist. In this he will be following the experience of the general practitioner of medicine. The media should when inoculated be at a temperature not below the temperature of the office. Preferably they should be at body temperature (37° C.) and the inoculated tubes or plates must be kept at 37° C. until sent to the bacteriologist. While to expedite the bacteriological examination of oral lesions it seems indicated to purchase the media ready made, and to send the tubes as soon as possible after inoculation to the professional bacteriologist for incubation, examination and report, still the author cannot too strongly emphasize that the actual collection of material and the inoculation of the media should be done by the dentist himself. There is no one else who knows quite so well the anatomy of the parts affected or the idiosyncrasies of conformation presenting themselves in the individual case. This crucial step in the work cannot satisfactorily, and should not, be delegated to anyone.

A point which requires preliminary emphasis is the need to take all reasonable precautions against contamination. This is particularly difficult in the mouth, because of the smallness and relative inaccessibility of the affected parts, because of the poor illumination of the field, and, finally, because of the omnipresence of saliva. If we find organisms on our culture we want to feel confident that their presence is not due to ignorance or negligence on the part of the dentist inoculating the media. It is assumed in the following descriptions that the practitioner is so familiar with elementary bacteriological technic that he will instinctively take the simple yet indispensable precautions to prevent contamination of the medium during its inoculation.

Finally, before entering upon the details, a word should be said relative to the interpretation of results. If, after taking adequate precautions against contamination we find bacteria present in the smear or on the culture, this constitutes valuable, dependable information. If, on the other hand the results of our examinations, microscopic or cultural, be negative, if we fail to establish the presence of bacteria, this information is far less valuable and significant. In the case of negative results there is always the suspicion that after all viable bacteria may still persist in the lesion. Concrete references will later be made to this very necessary attitude in interpreting results.

THE PERIAPICAL REGION.

From the strictly dental stand-point, the clinical applications of bacteriology find their widest employment in the examination of the periapical region with the tooth *in situ*. The information so gained may be useful in diagnosing the presence of infection, in controlling the treatment, and in coöperating with the physician who may want not only to determine the presence or absence of infection, but also to secure material for the preparation of auto-genous vaccins for extra-oral secondary conditions. The routine followed in this connection in the Department of Operative Dentistry in the School of Dentistry, University of Pennsylvania, is first to make a direct microscopic examination and then to follow this with a cultural examination. Cevéy¹ urged in addition to a culture, a direct microscopic examination of a smear from the canal, with the idea of demonstrating microörganisms which fail to grow on artificial media.

Before giving the details of the direct microscopic examination it will be well to orientate oneself historically and medically. With the reintroduction of antiseptic surgery (in contrast with aseptic) in the management of infected wounds in the World War, came the need for some method which would keep the surgeon informed as to the progress of his treatment. This need was met by the direct microscopic examination of exudates from the wound. The technic of this method is most succinctly given by Dumas and Anne Carrel². Smears from different parts of the wound are made on clean glass slides, dried, fixed by heat and stained for thirty seconds by phenol thionine (Thionine 1 gm., glacial phenol, 1 gm., 95 per cent alcohol,

¹ Schweiz. Vrtljhrschrt. f. Zahnk., 1917, 27, 221.

² Pratique de l'irrigation des plaies dans le méthode du Dr. Carrel, Paris, 1917, p. 21.

10 gm., and 100 cc H₂O). This is washed off in water; the slide dried and examined with the oil-immersion lens. In counting the field of the microscope oc. 3, obj. 1/12 imm. is the unit field. First count the number of bacteria in 10 fields in one part of the slide, then in 10 fields in another part of the slide and then in another 10 fields in a third part, then calculate the mean number of bacteria per field. These authors point out the clinical significance of these counts. Disinfection should lower the number. If this remains stationary many days or if it increases, the surgeon should examine the wound to seek the causes of the arrest of sterilization. If one finds only isolated cocci or diplococci, if one finds only 1 per 4 or 5 fields and if this result is verified on two or three successive examinations at one- or two-day intervals, disinfection is practically (surgically) complete, *i. e.*, the natural defensive forces of the patient will take care of the few surviving bacteria.

Carrel and Dehelly¹ call attention to another change in the nature of the exudate which occurs with the progress of disinfection. At first, polymorphonuclear leukocytes predominate but, when the microbes begin to become rare, the mononuclears increase in number. In the smears from root canals, polymorphonuclears often at first are abundant. In the course of treatment with dichloramin-T their number diminishes, but no other type of cell comes to take their place.

Carrel and Dehelly are very emphatic that the microscopic examination of the exudate in war wounds in spite of its lack of scientific precision does afford clinical data which are indispensable for the control of the treatment. The method of microscopic examination of smears from root canals, given in outline below, is merely an adaptation of the method described by Dumas and Anne Carrel. The dentist who visualizes the difficulties of root-canal work in true perspective and who has given the direct microscopic examination on adequate trial will be convinced of its utility.

Microscopic Examination of Smear.—1. A thin, broad smear is made on a clean slide with the dressing that has been sealed in the canal approximately twenty-four hours, if the dressing appears to indicate a clinically satisfactory condition. The material smeared must be exudate which has seeped into the canal. If the dressing or canal be dry, if the exudate be infinitesimal or absent, satisfaction cannot result. If the dressing be discolored badly or even faintly odoriferous, treatment should at once be reinstituted without taking the time required for the microscopic examination. If much

¹ *Le traitement des plaies infectées*, 2d ed., Paris, 1917, p. 141.

blood is mixed with the exudate, the examination will prove unsatisfactory because of dilution and obscuration.

2. Allow such a smear to dry in the air, fix by rapidly passing through a Bunsen flame three times, and,

3. Stain with methylene blue for thirty to forty-five seconds.

4. Wash off stain, blot dry, put on drop of cedar oil and examine with oil-immersion lens (at least 1.9 mm. objective).

If bacteria of any type are recognized, the treatment is reinstituted without proceeding to cultural methods. If no bacteria can be found, then the cultural examination is undertaken according to one of the methods described below. It has been found in this case, as is universally recognized in the case of diphtheria and pulmonary tuberculosis, that cultural methods are the more delicate and often will give a positive result where the result of the direct examination of the smear was negative. An inspection of the records of 937 cultures made in connection with the regular student root-canal work at the University of Pennsylvania gives the following result. Before each of these cultures was made, there had been a direct microscopic examination of a smear made from the canal dressing, which microscopic examination had failed to reveal bacteria. This negative result was confirmed by 467 of the cultures, and contradicted by 470 of the cultures. In other words, of two negative microscopic examinations, one will be confirmed and the other contradicted culturally. These figures represent only an approximation. The direct microscopic examination, preceding the culturing, was made by students, *i. e.*, by novices, and the author is certain that in many instances a smear was pronounced negative, which would be positive to a more expert eye.

There is a widely operative principle of psychology to the effect that what we earnestly desire we confidently expect. Now the practitioner earnestly desires sterility in his root-canal work and so does the student and the confidence of the latter is the less tempered by experience. If, on the contrary, the examiner approach the smear with an earnest desire to find bacteria, the percentage of positive smears will rise, but the percentage of positive results among the cultures taken subsequently to negative smears will drop.

The direct microscopic examination in case of periapical infection can be frequently completed in two minutes and never should occupy more than five. It is done while the patient is in the chair, and in case of a positive result one can at once return to the treatment without the delay entailed in the cultural examination. Not only is the time, but what is at least quite as important, the continuity of the treatment is saved.

Although the author feels that this microscopic examination should wherever and whenever possible, be followed by the more delicate and reliable cultural examination, still it must be recognized that it may not in all circumstances be feasible to go through this second step. In such cases, the direct microscopic examination alone may be used and it will prove to be at least an appreciable improvement over current visual and olfactory tests. Many times a dressing, which grossly appears inoffensive, will show microscopically the incontestable presence of bacteria.

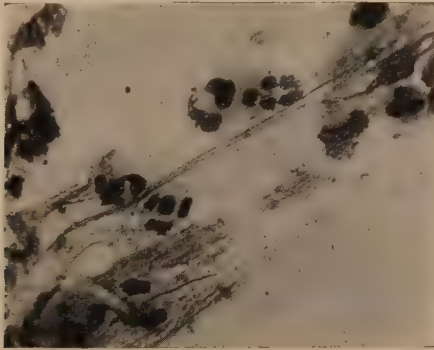


FIG. 88.—Smear from root canal. Stained with carbol fuchsin. The photograph was taken from a field at the margin of the smear. Polymorphonuclear leukocytes are conspicuous. These cells in some areas of the slide were so numerous and so closely packed together that it would have been almost impossible to recognize scattered bacteria. In such a field as here represented, the conditions are favorable for the recognition of bacteria. Nevertheless, none were found microscopically here. In such cases the more delicate cultural examination almost invariably reveals the presence of bacteria.

Sometimes, especially where dichloramin-T has been used, the smear will show innumerable "pus" cells, although thorough search will fail to reveal a single indubitable bacterial cell. Such a condition, *i. e.*, a purulent exudate, although not incompatible with sterility, should be regarded with suspicion as almost always the results of culturing such cases have been positive (see Carrel and Dehelly (1917, *supra*). (Fig. 88.)

An objection has been offered to the writer *à propos* of this microscopic examination which may be stated as follows: Granted we do see bacteria in the smear, how do we know they were alive when removed from the canals? One answer is that in every case where cultures have been made from cases presenting recognizable bacteria these cultures have proven positive. Radziewsky¹ made

¹ Ztschr. f. Hyg., 1901, 37, 1.

an observation which is pertinent: bacteria which are undergoing dissolution soon lose their colorability by methylene blue.

It seems just to state, as a principle of surgery that no nidus of infection which can be eliminated should be tolerated. Modern root-canal work seems to be based on the assumption, which is still *sub judice*, that in some selected instances the root canal, the dental tissues and the periapical tissues of pulpless teeth can be rendered and maintained sterile.¹ It is with the hope of gaining a reliable insight into the progress of the treatment toward this end that recourse is had to bacteriological examination. It is well to realize that this is not infallible. The Wassermann is sometimes negative in the presence of syphilis and the Neisserian diplococcus cannot always be found in chronic gonorrhea. The value of bacteriological examination in root-canal work is distinctly limited. Bacteria have been isolated from teeth whose canals were filled only after a negative culture. A negative culture does not *ipso facto* give the dentist warrant to proceed to the definitive occlusion. A positive culture, however, means emphatically that the canals are not yet ready for filling. In this lies the chief usefulness of bacteriological examination in root-canal work. A little reflection will convince one that to know when not to fill is information not to be lightly valued.

The author does not wish to deprecate the usefulness of bacteriology in this connection. The difficulties inherent in the histology and anatomy of the parts involved, alone would convince the impar-

¹ One important factor which determines the rate of chemical and physico-chemical reactions is the surface or area of contact between the reacting substances. The smaller the area, the slower the rate; the larger the area, the more rapid the rate. This rule holds true of disinfection, at least by chemical agencies. There must be effective contact between the disinfectant and the infected substance. The larger that effective contact, the more rapid and complete will be the destruction of the bacteria. In the case of the surgical treatment of war wounds the surface infected and the surface to which the chemical can be applied are practically identical. Consequently by proper means the bacteria can be so reduced in numbers that the natural body defenses can take care of any few surviving germs and the wound may be closed; *i. e.*, the wound is surgically "sterile," not necessarily bacteriologically or absolutely sterile. It still remains an open question whether even under the very favorable condition of a large area of contact under which the war surgeon worked, it is possible to secure bacteriological or absolute sterility. Pelicard and Philip (reference incorrectly given by Carrel and Dehelly) and Debeyre and Tissier (*Compt. rend. Soc. de chir.*, March 20, 1917) consider the absolute chemical sterilization of wounds impossible of attainment. On the other hand, Vincent (*Jour. Exper. Med.*, 1917, 26, 88) asserts this possibility on the basis of 7 bacteriologically sterilized wounds out of 20 infected bases.

Turning to the problem of the dentist in treating periapical infection *via* the root canal, we must recognize that the difficulties are greater. The surface to which the disinfectant can be applied is relatively insignificant in comparison with the volume of the infected tissues. Consequently in the absence of conclusive proof that the periapical tissues can be sterilized (surgically or bacteriologically), it seems reasonable to exact a negative culture before filling the canal.

tial operator of the desirability of a simple refinement which will reveal frequently the failures and shortcomings of his treatment.

The question has come up, how long should cultures be incubated before a reliable report can be obtained for the dentist? M. L. Rhein, in a personal letter to the writer, states that "we do not give up expectation of obtaining a culture inside of twelve days. Very frequently I find the first evidence of *Streptococcus viridans* showing as late as the seventh day." Crane¹ reports 1 case in which a streptococcus growth appeared on ascitic fluid medium thirteen days after inoculation and 1 case in which growth appeared eight days after inoculation on Besredka's egg broth. With these two exceptions, however, no growth appeared in either of the above media after the third day. The author recalls once, in testing the sterility of a streptococcus vaccine, when the growth did not appear till the seventh day. Growth was obtained in all but 5 of the 470 positive cultures, mentioned above in connection with the routine student root-canal work, within forty-eight hours. These 5 exceptions showed growth between the second and the fifth day of incubation. This delay can, however, be of only infrequent occurrence and in the eyes of most clinicians is too long for practical purposes. At the Dental School of the University of Pennsylvania, as a routine measure, a tube of bouillon is inoculated directly with a paper point and incubated until the next morning, when unless unmistakable growth be present, 2 or 3 loopsful are transferred to the surface of a very moist agar slant. This is incubated until the next morning. Consequently the report is sent from the laboratory to the operative clinic not later than forty-eight hours after the culture was first taken. The incubation should not be less than this, and might be extended with some advantage, *i. e.*, a higher incidence of positive results.

CULTURING THE PERIAPICAL REGION, WITH THE TOOTH IN SITU.

Method No. 1.—Through the Root Canals.

- (a) Isolate tooth with rubber dam.
- (b) Sterilize coronal surface of tooth.
- (c) Remove filling, temporary or otherwise, with sterile instruments.
- (d) Remove filling or dressing in the root canal with sterile instruments.

¹ Dent, Cosmos, 1918, 60, 1093.

(e) Mechanically cleanse the canal and finally dust its surface with sterile paper points moistened slightly with sterile distilled water or sterile physiological sodium chlorid solution.

(f) Dry the canal with sterile, dry paper points.

(g) If any moisture oozes into the canal from the periapical tissues, as is very frequent when dichloramin-T is used as the sterilizing agent¹ this may then be absorbed with a dry sterile paper point, which is then removed from the canal and dropped into an appropriate medium; or

If no liquid enters the canal from the periapical tissues, a sterile, fine broach or pick may be passed through the canal to collect the material for inoculation. If a liquid medium be first inoculated, subinoculations after twenty-four hours' incubation at 37° C. may with advantage be made onto the surface of a solid medium. It is often very much easier to recognize a growth on a solid than in a liquid medium.

Method No. 2.

More elaborate precautions are recommended by Rickert.²

The canals are first opened large enough to be readily accessible. The canal walls should be cleansed with alcohol or hydrogen dioxid. The treatment is introduced on an aseptic paper or cotton point of a length not to exceed two-thirds the length of the canal, above this toward the occlusal orifice place a short section of the dry thickened end of a sterile cotton point; then above this place cotton moistened with sandarac varnish. The cavity is next sealed with either cement or temporary stopping. In taking the culture, the tooth and adjacent teeth are isolated, dried and treated with tincture of iodine. The temporary stopping is removed and the cavity moistened with iodine; the sandarac varnish stopping is then removed and this portion cleansed with alcohol. The last dry pledget is then removed and with a barbed broach the dressing to be cultured is carefully withdrawn; it is seized just above the point of contact of the broach with sterile cotton pliers; the broach is released, the outer end released from the broach, cut off to the pliers and the remaining part of the point, which is the apical end, is introduced into the culture media. In this way the upper part of the canal has been made safe for the withdrawal of the dressing to be cultured. The procedure at first looks tedious and difficult, but is less so than it would appear.

¹ Prinz: *Dent. Cosmos*, 1918, **60**, 1071.

² *Jour. Nat. Dent. Assn.*, April, 1922, p. 300.

Method No. 3.

(a to f) (Exactly as in Method No. 1.)

(g) A small drop of sterile glucose bouillon or other liquid medium is run into the canal.¹

(h) Seal canal aseptically.

(i) After twenty-four hours open canal aseptically as indicated above (Method 1; a, b, c, d), and with sterile broach, spick, or needle, collect some of the material for inoculation.



FIG. 89.—Aspirating needle to be attached to Elgin casting machine. The aspirating needle is made by soldering a cupped disk of 30-gauge platinum plate about $\frac{3}{8}$ inch from the end of the platinum point of a Berlin abscess syringe. The disk acts as a platform upon which to carry temporary stopping to seal automatically the cavity when the needle has been introduced into the canal. (Crane.)

Method No. 4.

(a to f) (Exactly as in Method No. 1.)

(g) Seal a specially adapted hollow needle into the enlarged mouth of the canal. (See Fig 89.)

(h) Connect needle with suction pump of about 12-pounds negative pressure. This is to draw tissue juices into the canal from the periapical region.

(i) With broach, pick, or needle collect some of this material for inoculation.

Method No. 5.

The following method has been proposed by Head.²

When the area of infection appears near the root tip of a tooth in

¹ LaRoche: The Relation of Bacteriological Findings in Relation to the Treatment of Infected Teeth, Jour. Allied Dent. Soc., June, 1918, p 155.

² Modern Dentistry, Philadelphia, 1917, pp. 93 *et seq.*

which the pulp is alive, the following method should be employed: The mucous membrane over the indurated spot should be anesthetized and a thin cantery plunged down to the bone. An opening should then be made through the outer plate of the alveolar process by a sterilized bone drill. At the end of two days the outer opening in the gum should be protected by a napkin, anesthetized, and cauterized again. The point of a small sterilized platinum-pointed glass syringe or platinum spear should then be inserted into the bony opening, and, with the same care previously mentioned, a drop of bloody fluid extracted and transferred to the blood-agar tube. This material will unquestionably contain the bacteria from the infected area that have gathered and grown during the two intervening days, owing to the lowered vitality of the wounded tissue.

Method No. 6.

This method is *a priori* less objectionable than any of the preceding ones, on the ground of the danger of accidental contamination from the dentin. It is particularly suitable where the tooth is serving as an abutment or bearing a crown. It is essentially the technic of root amputation. It is questionable how reliable this method or No. 7 or No. 8 below, is in the case of teeth surrounded with "pyorrhea" pockets.

(a) Radiographically determine position of apex.

(b) Sterilize overlying mucosa, *e. g.*, with tincture of iodine or brilliant green and crystal violet (1 per cent of each in 50 per cent alcohol).

(c) Isolate area with cotton rolls to minimize danger of salivary contamination.

(d) Under local anesthesia make a curved incision with a sharp scalpel through the soft tissues to the bone.

(e) Reflect muco-periosteum.

(f) With appropriate bur, cut through alveolar bone to the tooth.

(g) With curette collect some of the periapical granulation tissue for inoculation. The material so collected should be subjected to a preliminary trituration. This may be done conveniently in the following manner: A small pinch of sea sand, washed and ignited, is added to a tube containing about 0.25 cubic centimeter of nutrient bouillon, and the whole sterilized. The infected(?) periapical tissue is added to such a tube. A long, sterile glass rod with its ends fire-polished is used as the pestle in grinding the tissue with the sand. The tube should be held nearly horizontally. Inocula-

tions onto appropriate media are made from the resulting suspension.

Canouse¹ does not curette, but introduces a capillary glass pipette deeply into the infected tissue and applies suction. The pipette may then be sealed in a flame and sent to the laboratory for inoculation. The pipettes are prepared as follows: A piece of 6 mm. soft glass tubing 6 or 8 inches long is plugged with cotton at both ends and sterilized in the hot, dry-air sterilizer. By the application of a flame to its middle section it may be drawn out into two capillary pipettes with sealed tips. This gives a pipette sterile on the inside, and the outside of the tip may be sterilized just before taking the culture by passing through the flame. Just before use, the sealed tip is broken off. Holmes² similarly used a glass pipette or syringe.

(h) Close operation wound.

*Method No. 7.*³

(a, b and c) (Exactly as in Method No. 6.)

A dental hypodermic syringe, which has been previously boiled and allowed to cool, is fitted with a needle, sterilized in the same way and finally passed through a Bunsen flame. A puncture is then made under local anesthesia through the gum and alveolar process down to the root tip, and an attempt is made to aspirate. The aspirated material, whether visible or not, is transferred to suitable culture media.

Method No. 8.

This is a modification of Method No. 6, considerably simplifying the procedure both for the dentist and the patient.⁴ From the stand-point of the bacteriologist, this is the method of choice, whether the tooth is to be tentatively retained or immediately extracted, if the drill actually exposes the periapical region.

(a, b and c) (Exactly as in Method No. 6.)

(d) Under local anesthesia, drive the sterile trocar attached to the right or contra-angle of the dental engine, through the tissues to the root apex. (See Fig. 90.) Not only must the trocar be sterile but the handpiece as well.

(e) Remove the twist drill.

¹ Western Dent. Jour., August, 1917, p. 20.

² Jour. California State Dent. Assn., March and April, 1918, p. 146.

³ Cohn, M. B.: Internat. Jour. Orthodont., 1918, 4, 36.

⁴ Coriell: Dent. Cosmos, 1918, 60, 1154.

(f) Through the trephine insert sterile needle to collect material for inoculation.

(g) Remove the trephine. The wound is so small that it requires no special attention.

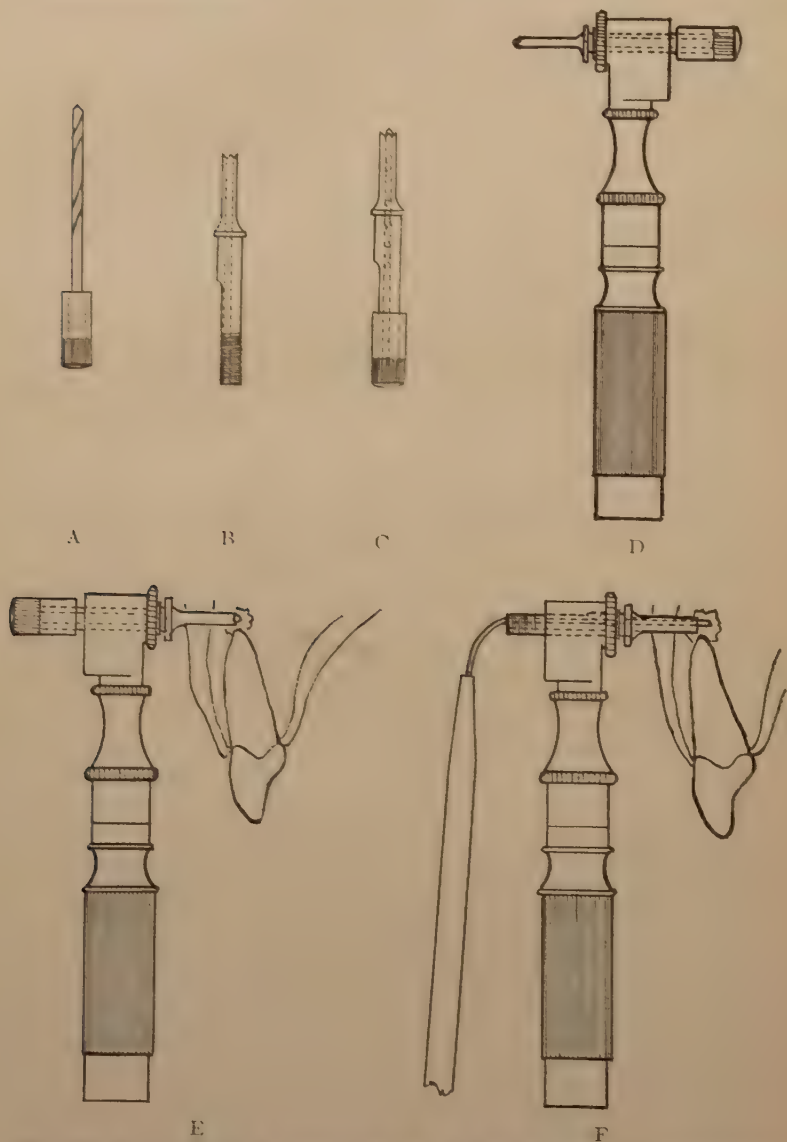


FIG. 90.—In detail. Fig. A represents the drill; B the trephine; C, the assembled trocar, and D, the trocar in the handpiece. Figs. E and F show the method of obtaining the culture.

The periapical region may be cultured after extraction. There may arise occasion for this when the dentist coöperates with the physician in cases of suspected oral focal infection. Cultures alone in this instance are indicated and smears for direct microscopic examination are superfluous. The work is done at the physician's request, who may desire to have an autogenous vaccinn prepared. This concerns the general systemic condition of the patient, and the dentist's liability ceases with the extraction of the tooth and the taking of the culture as requested by the physician. The method given above as No. 8 is also suitable for use immediately before extraction, and seems less open to the objection of possible contamination and therefore preferable to any of the four following procedures.

Method No. 1.—Culture from the Socket.

- (a) Cleanse and spray the whole mouth.
- (b) Remove deposits from the tooth to be extracted.
- (c) Dry the crown and exposed parts of this tooth and its neighbors.
- (d) Paint these parts and the adjacent soft tissues with tincture of iodine or brilliant green and crystal violet, 1 per cent of each in 50 per cent alcohol.¹
- (e) The free gingival margin is sometimes electrically cauterized.
- (f) Isolate the tooth and its neighbors with sterile cotton rolls.
- (g) Cut the tooth free from the gingivæ using for example one of the instruments for the surgical treatment of "pyorrhea"² or the peritome of Mueller.³
- (h) Paint the cut edges as in (d) above.
- (i) With forceps with absolutely sterile beaks remove the tooth, taking care to prevent the saliva or the tongue from entering the socket.
- (j) With sterile curette remove some scrapings from the depth of the socket or with a sterile cotton swab, and with the material so collected make the inoculations.

Method No. 2.—From the Apex to the Root Itself.

- (a to i) (Exactly as in Method No. 1.)
- (j) Place tooth in sterile Petri dish or wrap in a piece of sterile

¹ Berwick: Jour. Dent. Res., March, 1920, p. 21; Jour. Infect. Dis., 1921, **29**, 537.

² Ziesel: Pyorrhea Extermination: Gingivectomy, Dent. Cosmos, 1921, **63**, 352.

³ Dent. Cosmos, 1923, **65**, 1036.

gauze, until the culture can be made. The interval before the inoculations should be as short as possible.

(k) If a sac is adherent to the tooth after removal from the socket, the surface of this sac may be cauterized with a broad spatula-like needle heated to redness. With another, pointed sterile needle the sac may be punctured through the cauterized area. A loop-needle, or one whose last free millimeter has been bent at right angles to the shaft, may be inserted through this puncture and twisted around within the interior of the sac. Upon withdrawal, inoculations may then be made upon appropriate media. The sterile, glass capillary pipette is well adapted to collect the material for the inoculations. It is stabbed directly through the seared area into the sac and suction applied.

Method No. 3.

The following method is applicable when the apex of the extracted tooth presents an adherent sac and when the filling in the root canal is absent or incomplete.

(a to j) (Exactly as in Method No. 2.)

(k) With sterile, heavy, bone-cutting or rongeur forceps snip off apical third or quarter of the root. Take care that neither part of the tooth flies from the sterile Petri dish or sterile gauze.

The tip of the root may be dropped directly into the culture medium,¹ or with sterile forceps pick up apical part of tooth, holding root canal toward operator.

(m) Through the root-canal into the adherent sac, pass a fine, sterile broach, apexograph, or pick; twist around within the sac.

(n) Remove and inoculate appropriate media.

Method No. 4.—In Case no Sac Adheres to Apex at Extraction.

(a to j) (Exactly as in Method No. 2.)

(k) A sterile loop may be used to collect a bloody liquid over the apical foramina or, in case the apex is dry by the time the cultures are to be made, the apical area may first be moistened with a loopful of sterile bouillon. Appropriate media may be inoculated with this material.

¹ Hartzell: Secondary Infections Having Their Primary Origin in the Oral Cavity, Jour. Allied Dent. Soc., 1914, 9, 166.

BACTERIOLOGICAL EXAMINATION OF THE PARIETAL PERIODONTIUM.

Microscopic.—So far as the writer is aware Mendel¹ is the only one who has attempted to recognize a "prepyorrhoeic" state by microscopic examination of the exudate in the gingival trough. Normally the exudate presents a leukocytosis with a differential count of 90 to 95 per cent polymorphonuclears, 4 to 5 per cent large mononuclears and 2 to 3 per cent lymphocytes. The phagocytic behavior of these cells is most significant clinically. Mendel reports that in young normal individuals there are 5 to 6 phagocytes per 100 leukocytes; that with the appearance of the hyperleukocytosis the phagocytic ratio rises to a mean of 50 to 60 and up even to 80 per 100; and, finally, in well-marked pyorrhoea alveolaris the ratio is rather low, *e. g.*, 9, 10, 15, 16, 18, 23 per 100 leukocytes. If the results of these observations were established as generally valid, the simple microscopic examination of the gingival exudate would prove to be of the highest clinical value. An hyperleukocytosis or a phagocytic ratio, ranging approximately from 50 to 60 or more would indicate incipient and even macroscopically unrecognized periodontal disease. Although Jenner's or Wright's blood stains are habitually recommended for phagocytic estimations, in this laboratory we have found Bordet-Gengou's toluidin blue, as suggested by Evans,² preferable. This stain is prepared as follows: 5 gm. toluidin blue (Gruebler); 100 cc alcohol; 500 cc water, and 500 cc of 5 per cent phenol. Filter after one or two hours. One part of stain is diluted with 2 parts of water for staining the smears. The filtration and dilution should occur just before use. It is applied only twelve to fifteen seconds.

Although the author has not attempted to examine pragmatically Mendel's statements, an observation has repeatedly come to his attention which suggests the usefulness of the direct microscopic examination of stained smears from the gingivæ. Among the laboratory exercises required of the dental student in bacteriology is the staining and examination of smears made from the mouths of other members of the class. In going over such preparations it has been found that conspicuousness of spirochetes or a larger number of polymorphonuclear leukocytes, than an isolated one here and there, is invariably pathognomonic of gingival irritation. The existence

¹ Ann. d. Odontologia, 1917, 2, 103.

² Hygienic Laboratory Bull. No. 134, Treas. Dept. United States Public Health Service, May, 1923.

of such lesions in most instances was unknown to the student and even unrecognized until after attention had been particularly called to them by the microscopic examination. Then it was found that the site, from which the material had been collected for the smear, was subjected to the trauma of salivary calculus, of a poorly adapted band or crown, of an unfinished gingival margin of a filling, or of abnormal occlusal relations. The point emphasized by these observations is that minute and clinically inconspicuous lesions were recognized easily by simple microscopic examination of stained smears, and that such lesions are widely regarded as being initial stages in refractory processes, often terminating in the exfoliation of the teeth.

Barrett,¹ in seeking the *Entamæba gingivalis* in "pyorrhæic" pus anticipatory to the application of the reputed amœbicide, emetin hydrochlorid, believes that the living, motile forms can most readily be recognized. A bit of the purulent contents of the pyorrhea pockets is mixed with a drop of slightly warmed physiological salt solution (the patient's saliva is admirable for this purpose—J. L. A., Jr.) on a warmed slide, covered and examined fresh and unstained with the 4 mm. objective. Bass and Johns,² for essentially the same purposes as actuated Barrett, made their microscopic examination as follows: (1) Thin, broad film of material from depth of pocket on clean slide; (2) dry in air; (3) fix by passing rapidly three times through Bunsen flame, (4) carbol-fuchsin; (5) wash off *at once*; (6) Loeffler's methylene blue 0.25 to 0.5 minute; (7) wash, dry, place on drop of oil, examine with oil-immersion lens. The writer has found this method universally useful in examining oral smears of all sorts. It is very simple and gives a beautiful field when correctly done. In such smears the entamœba appears as a roughly rounded cell, often slight larger than a polymorphonuclear leukocyte. The cytoplasm is vacuolated, bluish to purplish in tint. In the cytoplasm are circular inclusions, in number varying from three or four to a dozen or more staining different shades of red.

At the present time, the chief clinical interest in determining the microbial content of "pyorrhea" pockets seems to lie in discovering whether the dominant type be spirochetæ or bacterial (Coccaceæ and Bacteriaceæ). Although the microbial factor in "pyorrhea" is probably secondary, it certainly is not negligible and the search for spirocheticidal or bactericidal solutions as adjuvants is not irrational.

¹ Dental Cosmos, 1914, 56, 948.

² Jour. Am. Med. Assn., 1915, 64, 553.

Many investigators, among whom, Kolle,¹ Kritchevsky and Séguin,² Seidel³ and Cavalié and Mandoul⁴ have reported an abundance of spirochetes in "pyorrhea" pockets. The therapeutic inference in these cases has been to use a spirocheticide, especially one of the organic arsenicals. On the other hand, if bacteria predominate, any of the iodine-containing formulæ or possibly one of the chinin derivatives⁵ seems indicated.

The relative preponderance of spirochetes or bacteria in "pyorrhea" pockets can easily be determined by making a thin, broad smear on a clean glass slide and staining according to the technic given by Bass and Johns for entamœbæ. Methylene blue alone will do well if one were dealing with bacteria alone, but it will bring out the spirochetes only very poorly. Eyre and Payne⁶ used Leishman's modification of Romanowsky's stain, followed by 0.1 per cent aqueous solution of methylene blue. They also used Gram's stain. Goadby⁷ recommends Giemsa's stain in the microscopic study of smears from periodontal disease. He points out a finding which may prove to be of some clinical significance in prognosis. A film showing cells of bone-marrow origin, *e. g.*, eosinophils and nucleated erythrocytes, "indicates that the disease process has penetrated to the bony tissues, and that the specimen has been made actually from cancellous bone."

CULTURING PERIODONTAL (PERIODONTOCLASTIC) INFECTIONS.

From the purely dental stand-point there seems at present to exist no clinical indication for the culturing of "pyorrhea" pockets. However, the dentist in his coöperation with the physician in cases of suspected oral focal infection may be called upon to take cultures from these conditions. When this is done intelligently, this phase of the dentist's duty comes to an end for the responsibility of selecting the constituent organisms, the preparation and administration of an autogenous vaccin for a systemic ailment, *e. g.*, an arthritis, rests with the physician. The technic of making such inoculations follows:

¹ Med. Klin., January 21, 1917, p. 59.

² Dent. Cosmos, 1918, **60**, 781.

³ Deutsch. Zahnhlk., 1919, H. **41**.

⁴ Compt. rend. Soc. de biol., 1921, **85**, 1068.

⁵ Bruhn: Morgenrothsche Chinin-derivative in der Zahnheilkunde, Ergebn. d. ges. Zahnhlk., 1920, **6**, 154.

⁶ Brit. Jour. Dent. Sci., 1910, **53**, 153, 195.

⁷ Diseases of the Gums and Oral Mucous Membrane, Oxford Med. Pub., 1923.

Method No. 1.

(a) Isolate tooth with sterile cotton rolls, to minimize danger of salivary contamination.

(b) Stroke the gingival wall of the pocket, in the direction from the apex of the tooth to the gingival margin, to "milk" out the grosser quantity of food debris, adventitious microorganisms, and exudate. With sterile cotton pledget wipe away this discharge.

(c) Paint gingival *margin* with tincture of iodine, or brilliant green and crystal violet (1 per cent of each in 50 per cent alcohol).

(d) (1) With appropriate sterile instrument (scaler or heavy platinum needle) collect material for inoculation from the very *depth* of the pocket; or

(2) With sterile capillary pipette collect a bit of the material. Dilute it in a flask containing a few cubic centimeters of sterile sodium citrate (1 per cent) solution; and transfer a few drops of this suspension with a capillary pipette to a number of deep tubes of semiliquid medium. The medium may be ascitic fluid and ordinary nutrient agar, plus preferably a small piece of fresh, sterile rabbit-kidney tissue.¹ This method is satisfactory for the cultivation of anaërobic, *e. g.*, Vincent's fusiform bacillus and spirochete.

Method No. 2.

Eyre and Payne² used the following procedure.

In collecting the pus, the patient was instructed to grasp the lip opposite the infected teeth with the forefinger and thumb of each hand and draw it away from the gums; in some cases a small roll of absorbent wool was packed into the sulcus between the alveolus and the lip (vestibulum). The gum margin was next wiped with a sterile swab of cotton-wool mounted on the end of the stick, and the gum itself dried with a second sterile swab. Then with another swab firm pressure was made on the gum over the root of the tooth. The first drop or two of pus that exuded was mopped up with a third sterile swab and the pressure continued, and the pus that next exuded was collected on still another sterile swab, or by means of a stout platinum needle. This pus was employed for the purpose of making coverslip films. Finally more pus was expressed in a similar way and used to inoculate a tube of nutrient media (citrated blood-agar).

¹ Noguchi: *Treponema mucosum* (n.sp.), a Mucin-producing Spirochete from Pyorrhea Alveolaris, Grown in Pure Culture, Jour. Exper. Med., 1912, **16**, 194.

² Brit. Jour. Dent. Sci., 1910, **53**, 153, 195.

INFECTIONS OF THE ORAL MUCOSA.

The bacteriological examination of the surface infections of the mouth, the gingivitis and stomatitis, for clinical purposes may be confined to the direct microscopic examination of one or more properly stained smears. The particular affection of most frequent occurrence to fall under this division is ulcero-membranous stomatitis or Vincent's infection of the oral mucosa. While the diagnosis of this condition by cultural methods would be a matter of weeks, and even then negative results would be unreliable, it may be diagnosed by direct microscopic examination of a slide in a few moments at most. It can most emphatically be stated that Vincent's infection can only positively be diagnosed by bacteriological methods. (See Plate IV.)

Reference to the technic has already been made above,¹ but as its greatest usefulness lies in this connection, it will be given in some greater detail. The slide must be dry and clean. The material may be scraped from the affected mucous surface with a swab, scaler, excavator, curette or other appropriate instrument. This material is smeared in a thin, even film over the slide, covering as large a part of the surface of the slide as possible. The beginner almost always makes the film too thick. This film is allowed to dry in the air, and is then fixed by passing rapidly through a Bunsen flame. This has slightly raised the temperature of the slide, and the temperature should return to that of the room before adding the staining solution. The first dye used is carbol fuchsin, as prepared for use in demonstrating tubercle bacilli. This is poured on and *immediately* washed off in running tap water. Then Loeffler's methylene blue is added and allowed to act for about thirty to forty-five seconds. Then it is washed off in running tap water. The tint of the film should be distinctly on the blue side of purple. The optimum tint can only be recognized by experience. The time mentioned above, thirty to forty-five seconds, is only approximate and it will likely be desirable to vary it in each individual slide, depending upon the thickness and nature of the film. If after the first exposure to methylene blue the film is still reddish, then a second application of methylene blue is indicated of ten to twenty seconds. Sometimes a third application is necessary. When at last the proper tint is secured, the slide is blotted dry, a drop of cedar oil is applied and the examination is made with the oil-immersion lens. In acute, uncomplicated Vincent's infection no "pus cells" are found. The spirochetes appear as delicate, wavy lines, sometimes forming a densely matted network. The fusiform bacilli are large,

¹ Bass and Johns: Loc. cit.

straight or often slightly curved rods, pointed at both ends, taking a blue or bluish tint and often containing a number of small, brilliantly red granules. In examining a case of suspected Vincent's infection, at least three or four slides should be made, collecting the material from different parts of the mouth. The author has repeatedly failed to find a convincing picture of Vincent's infection in the first or second slide examined, only to find overwhelming evidence of this condition in the third or even fourth slide. The time element is negligible, for the entire preparation and examination of each slide need not occupy over two minutes.

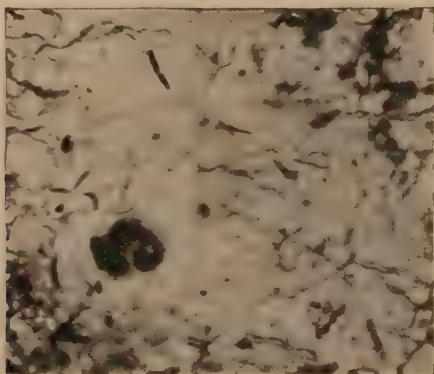


FIG. 91.—Smear from mouth showing Vincent's organisms persisting after the mucosa had grossly returned to normal. This picture is indistinguishable from that obtained when the clinical symptoms were completely developed.

Bacteriological methods should be used not only for the diagnosis of Vincent's infection, but should be employed to determine the progress of the treatment and when the treatment may be stopped. This disease usually yields readily to appropriate therapy and the mucosa may have apparently returned to normal, although microscopically abundant spirochetes and fusiform bacilli can be demonstrated. (See Fig. 91.) If the treatment be suspended while these organisms persist, even though grossly the mucosa is normal, there will be a recurrence. Especial attention should be given to seeking the organisms in out-of-the-way, minute foci about the mouth, such as an isolated pocket, an interdental space afflicted with a poorly fitting band or crown, or filling with roughened gingival margin, or the area distal to the last molar. Organisms persisting in such foci will quickly reinvade the entire mouth after premature cessation of treatment. In brief, before voluntarily releasing the patient, microscopic examination from a number of parts of the mouth on several successive days should prove negative. 2

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